Factors affecting dry matter yield and pyrethrin content in pyrethrum in Tasmania

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Abstract

Pyrethrum is a major crop in Tasmania, Australia. The lack of predictability in flower dry matter (DM) production and therefore crop yield is one of the biggest challenges for the industry, with large variation in dry matter yield currently occurring between production sites and seasons. If the industry is to expand, consistency in production is required to ensure crop production matches the volumes contracted for delivery to the market. Knowledge of the main factors affecting DM accumulation under commercial production conditions is required to develop crop management strategies capable of delivering consistent, high yields. The specific objectives of the studies reported in this thesis were to investigate the effect of: (i) light interception; (ii) plant density; and (iii) water stress on DM yield and pyrethrin content of pyrethrum.

The indirect measurement of light interception in pyrethrum using crop green leaf area and volume was used to assess the relationship with flower DM yield and pyrethrin content. Strong relationships were identified within sites and seasons, but not across all seasons and sites assessed in the study. As there was no clear relationship between light intercepted and pyrethrin yield, other factors could be involved. It was hypothesised that differences could be related to variation in rate of crop development, plant density and drought stress, which can occur during the later stages of flower development in Tasmania.

Plant density was shown to have a large effect on DM partitioning in a field trail conducted in north-western Tasmania in 2008-09. The relatively large leaf, stem and flower DM yield per plant at low (6 plants/m^2) plant densities was compensated by the increase in plant numbers at high (44 plants/m^2) densities. Variation in plant density is therefore likely to affect light interception. There was, however, no difference in the concentration of pyrethrin, the active ingredient extracted from the pyrethrum flowers, with plant density. No significant differences in DM accumulation were found between pyrethrum genotypes and there were no density x genotype interactions.

Drought stress was investigated by conducting pot trials under controlled conditions. Treatments included 3, 4 and 5 day watering intervals applied at early flower

maturity stage 2 (FMS 2), mid (FMS 4) and late (FMS 6) flowering stages for either short (10 days) and long (20 days) durations. Plants watered on a 3 day interval tended to have lower (less negative) leaf water potential and displayed no visual signs of water stress. The FMS stage that water stress treatment was applied affected plant and flower dry matter yield, but watering interval or duration of stress treatment had no significant effect. Plants produced greater DM when drought stress was imposed during early compared with late flowering. Plant pyrethrin content, measured as pyrethrin concentration in the harvested flowers, was not affected by any of the drought treatments. Consequently, it was concluded that plants grown in field conditions are likely to be able to recover from mild drought stress, particularly during early flowering, but yield will be reduced if plants are stressed at later flowering stages.

This thesis has provided information to the pyrethrum industry on plant growth in response to crop management strategies and has highlighted the complexity of processes determining crop dry matter and pyrethrin yields.

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Dedication:

Achieving a Masters by Research in Agricultural Science is historic for my immediate family who until recently have been subsistence farmers in the rural Siroi Community of Papua New Guinea. Therefore, firstly and foremost, I dedicate this thesis to my beloved parents, Mr Kubo Kud Kessom and Mrs Dabi Lileb Kud and family. Unfortunately my beloved daddy has passed away but I am very grateful that my old mother is still alive and able to see me achieve this level of education and progress in my scientific profession.

I also dedicate this achievement to my immediate clan and community of origin: Warit Clan, Kumisanger Village and Siroi area communities of Astrolabe Bay Local level Government in Saidor District, Madang Province, in Papua New Guinea.

Table of contents

Chapter 1. General Introduction

1.1 Pyrethrum

Pyrethrum *(Tanacetum cinerariaefolium L.)* is a perennial herbaceous species that grows to a height of 60 to 80 cm and produces daisy-like white flowers. The species is grown commercially for production of pyrethrins, a natural insecticide. Over 90% of the total pyrethrins are found in the secretory ducts and oil glands of the ovary (Chandler 1951; Gnadinger and Corl 1930; Head 1966b; Martin 1934). The oil glands are found externally on the surfaces of the ovary wall and the corolla while the secretory ducts are present within. The oil glands are also found on the surfaces of leaves, stem, leaf petioles and flower stalks and the secretory ducts in the roots, stem and leaf tissue (Zito *et al.* 1983). The increase in flower pyrethrin content has been closely related with the development of oil glands in the flowers (Bhat and Menary 1979). When the pyrethrin concentration reaches its maximum the flowers are harvested and dried before pyrethrins are extracted. The natural insecticidal property of the pyrethrum plant to kill and repel insects was first discovered in pyrethrum species in China in first century AD during the Chou Dynasty (Van Rijn 1974).

While the insecticidal properties of the species have been known for a long time, pyrethrum has only a relatively short history as an intensively cultivated commercial crop. The first major commercial planting was done in Yugoslavia prior to 1914. Thereafter Japan was the main producer until 1939 when Kenya and other East African countries took over after the second world war (Mkawale 2001; Van Rijn 1974). Several attempts have been made to grow pyrethrum as a commercial crop in different parts of Australia between 1890 to 1964 (Bhat and Menary 1984a). Pyrethrum was first introduced into Tasmania and a crop improvement program begun in 1978. The commercial production of the crop started with selected clones from the program in 1984 (Bhat and Menary 1984b) and the crop is now well established and commercially viable in Tasmania. While it is difficult to explain the reasons for early failure of the crops in other regions in Australia, the introduction of planting materials of inferior varieties resulting in low flower yield, diseases and lack

of adequate knowledge of the crop may have been some of the contributing factors (Bhat and Menary 1984a).

The total dry flower production worldwide was estimated to be 18 000 tonnes in 1995 (Wainaina 1995), valued at US$7500 million (Elliot 1995), and is likely to have grown significantly since that date. Tasmania is currently the world's largest pyrethrum producer, and the industry has potential to expand. There have been significant advances in the broadening of usage pattern of pyrethrin in recent years, including use in organic production systems, creating increased demand for the product. As a relatively undeveloped crop species, significant potential exists for increasing production efficiency and flower dry matter yield to meet projected increases in global demand.

1.2 Flowering

Flowering in pyrethrum in Tasmania commences during winter, with a minimum vernalization requirement for flower initiation of two weeks at 6 ˚C or three weeks at 12 ˚C under short days and day temperatures of 20 to 30 ˚C, with 18 ˚C night temperature demonstrated to be insufficient for vernalization (Brown and Menary 1994a). The flower head of the plant is produced at the terminal of the primary and lateral flowering stems. The pyrethrum flower head is a compound inflorescence consisting of two floret types; the disc florets with yellow corollas in the centre of the head and ray florets with white corollas form the head's outer rim (Bhat 1995).

The compound flower of pyrethrum opens in stages. Firstly the ray petals unfold and then the florets open sequentially from the margin to the centre, with a single whorl of florets opening each day (Chandler 1951). Once the disc florets are open, flower colour begins to fade and seed-set takes place if the florets have been fertilized (Head 1966b). No further flower buds are initiated in almost all crops when the flowers reach maturity (Faber 1980; Tattersfield 1931). Flower development is divided into eight maturity stages, which was first described by Head (1966a) and later revised by Potts and Menary (1987) (Table 1.1).

Table 1.1. Flower maturity stages defined by Potts and Menary (1987) that are used commercially in Tasmania

Stage		Description
FMS 1		Well developed closed buds
FMS 2		Ray florets emerged from bud
FMS 3		1/3 of diameter of disc with florets open
FMS 4		1/3 to 2/3 of diameter of disc with florets open
FMS 5		Between 2/3 and all of the disc with florets open
FMS 6		All florets open and up to half the disc area brown
FMS 7		Ray florets dry, disc florets intact but with little colour (late-over-blown)
FMS 8		Petal of disc florets fallen, stems dry 1cm below flower, seed mature

The optimum time for flower harvesting in Tasmania is between flower maturity stage (FMS) 4 to FMS 6 (Faber 1980; Potts and Menary 1987). The mature pyrethrum plant produces a large number of flowers and will have variability between flowers in FMS at any point in time, so the flower maturity index (FMI) is considered to be the best indicator of optimum harvest time and is determined on a flower population basis in crops (Boevink 1991; Chung *et al.* 1994; Faber 1980). FMI is calculated from a sample of flowers collected from the crop, and is expressed as the sum of individual FMS of 100 flowers. FMI values in the range 100 to 800 therefore correspond to average FMS values for flowers in the crop in the range 1 to 8. Flower maturity, when assessed at a crop level using FMI, was reported to have increased steadily over the spring and summer until late-summer when the entire crop consisted of stage 8 flowers and an FMI of 800 (Faber 1980). Pyrethrin accumulates as the flowers develop but reach a maximum within a crop between FMI 400 to 600 before declining as flowers mature and seed development proceeds (Bhat 1995; Bhat and Menary 1984a).

1.2.1 Pyrethrin

The commercial product from the pyrethrum crop is the insecticide referred to as pyrethrin, a product extracted from the dry, ground flowers of the pyrethrum plant. It is one of the most widely used plant derived insecticides (Wainaina 1995). The effect of the pyrethrins on insects is threefold. At very low concentration the insects are repelled, while higher concentration results in a 'knockdown' effect followed by death in longer exposures (Elliot 1969). There is a very slow building up of resistance to pyrethrin in the insect population and the product is non-poisonous for mammals and has no residual effects (Casida and Quisad 1995).

The pyrethrin insecticide extracted from pyrethrum flowers is a mixture of six related compounds. On a dry weight basis a pyrethrum inflorescence contains the majority of the plant's active compounds, reported in the range 1 - 2% pyrethrins (Casida and Quisad 1995; Fulton 1998; Sastry *et al.* 2001). The pyrethrin extract generally consists of a combination of six esters derived from two acids and three alcohols (Table 1.2), although variation in components changes with country of origin (Bhakuni *et al.* 2007). The six insecticidally active compounds are pyrethrin I,

cinerin I, jasmolin I, and pyrethrin II, cinerin II and jasmolin II, with pyrethrins I and II present in higher concentrations (Crombie 1995; Head 1966a). A typical extract contains the pyrethrins, cinerins and jasmolins in the proportion 10:3:1 (Crombie 1995).

Table 1.2 The two organic acids and three alcohols which give six pyrethrins with the proportion in which they occur in Kenya (Parleviet and Brewer 1971)

Alcohol	Organic Acid	
	chrysanthemic acid	pyrethric acid
Pyrethrolone	pyrethrin 1 (35%)	pyrethrin 11 (32%)
Cinerolone	cinerin 1 (10%)	cinerin 11 (14%)
Jasmolone	Jasmolin 1 (5%)	Jasmolin 11 (4%)
	Pyrethrin 1 Fraction (50%)	Pyrethrin 11 Fraction (50%)

Pyrethrin levels are often reported as pyrethrin content, which is defined as the % pyrethrin per unit of dry matter. Pyrethrins accumulate in the flowers as they mature, typically with a 10-fold increase from 0.4 mg in closed buds at FMS 1, to 4.0 mg in mature flowers at FMS 8 (Berkley *et al.* 1938; Head 1966b; Martin and Tattersfield 1931; Parleviet 1970).

The pyrethrin is extracted from the dried, ground and then pelletised flowers using organic solvents such as hexanes to produce oleoresin concentrate containing 20-30% pyrethrins. In Tasmania, a pyrethrum refining process uses carbon dioxide in a super-critical-fluid state has been developed, thus avoiding exposure of the product to heat and risk of explosion or flashing of the solvents (Carlson 1995).

1.3 Tasmanian pyrethrum industry

The initial introduction and research into pyrethrum as commercial crop for farmers in Tasmania began in 1978 at the University of Tasmania (Bhat and Menary 1984b; MacDonald 1995). Commercial production of pyrethrum using varieties developed from research at the University of Tasmania commenced in 1984 when the Commonwealth Industrial Gases (CIG) Pyrethrum entered into an agreement with the Tasmanian Government and University of Tasmania (Bhat and Menary 1984a;

Macatta 2001). Botanical Resources Australia Pty Ltd (BRA) purchased the pyrethrum production and processing business from CIG in 1996 and took over management of the pyrethrum industry.

Production of pyrethrum in Tasmania is based in the Northwest Coast region. BRA contracts more than 220 farmers to grow pyrethrum crops. The land area under cultivation has increased from 750 ha in 1996 to over 2 200 ha in 2001. Pyrethrum crop production is managed on a contractual basis, with BRA organising planting and harvesting utilizing specialised machinery and provision of technical advice to growers contracted to produce the crop on their farms. The Tasmanian pyrethrum industry has recently expanded its production base to mainland Australia and Papua New Guinea.

The expansion in pyrethrum production has been driven by increasing global demand for pyrethrins. Refined pyrethrin oil is used as the key ingredients in domestic and industrial pest control formulation. Fast acting and broad spectrum, the insecticide is relatively safe to use everywhere from homes to broad-scale spraying operations. It is also one of the few insecticides approved for use on organic farms in Europe, the USA and Australia.

Tasmania is currently the world's largest pyrethrum producer supplying as much as 80% of the total world market while east African countries, such as Kenya supply the rest (Macatta 2001). Most of the pyrethrin produced at BRA's Ulverstone plant is exported to USA, while around 15% is shared between Germany, Holland, Spain, Italy, Hong Kong, Singapore, Korea, Japan, India and New Zealand (Macatta 2001).

1.4 Tasmanian production environment

1.4.1 Climate

Tasmania is an island state situated off the south east corner of mainland Australia. The state is located 42° south of the equator and has a temperate climate with cool winters and a mild summer, but within the state climatic conditions vary significantly between regions. The average maximum summer and winter temperatures are about 19 ˚C and 11 ˚C respectively, with an average minimum winter temperature of 4 ˚C. The average annual rainfall varies from 1400 mm in the west and north eastern

highlands to 500 mm in eastern parts of the state (Gentilli 1972). The average monthly rainfall ranges from 40 mm in January to 105 mm in July with rainfall generally higher in winter, but summer rains are still significant.

Pyrethrum is produced mainly in the north of the state. Much of the remaining regions in the state have been classified as unsuitable for pyrethrum production although crops have been established successfully in the Coal River and Derwent River valley regions in the south east (Fulton 1998). The main environmental factor thought to limit suitability of certain regions for pyrethrum production is frost, particularly spring frosts that may freeze developing buds.

1.4.2 Soils

The predominant soil type in the pyrethrum production region is the Ferrosol, locally known as Krasnozems. The soil is derived from the extruded volcanic deposits of volcanic rock such as basalt. The characteristics of this soil are described as red to brown, acid, strongly structured clay soil ranging in depth from 1 to 7 m (Isabel 1994). The soil generally has deficiencies of N, P, K, Ca, Mg and various micro-nutrients. The available soil moisture tends to change slightly with soil cropping history and depth (Bridge and Bell 1994). The soil is also known to maintain good structure under intensive cropping, with high initial infiltration rates and available moisture. Continuous cropping on this type of soil will result in significant reductions in water infiltration rates, bulk density, organic carbon, aggregate stability, water holding capacity and crop water extraction (Bridge and Bell 1994).

1.5 Intensive pyrethrum production system in Tasmania

Pyrethrum production in Tasmania is seasonal and the state is the only pyrethrum growing area in the world where production is fully mechanized. Crop establishment is based on direct seeding into a finely tilled soil at a sowing depth of 2.0 to 2.5 cm from August to December. Flowering of pyrethrum in Tasmania occurs during spring and early summer when light integral is increasing compared to winter as a result of less cloud cover and longer day length. The harvesting of the crop is first done 15 to 18 months after sowing, with most crops having a commercial life of four annual harvests before termination. Harvesting is done in late December using a mechanised

system known as cutter-rowers. The flowering stem is cut a few centimetres above the ground level and windrowed for field drying for approximately 12 to14 days depending on sunlight hours, humidity and temperature. Once the windrows are dried, modified combine harvesters are used to cut, grind and separate the achenes containing the pyrethrin from other plant material which is returned to the field as trash (Macatta 2001).

In general, commercial crops are terminated after four annual harvests because yields are reduced significantly by this stage in most crops (Natural Resources Environment 1998). The highest yields are nearly always achieved in the first harvest and yields progressively decline in harvests 2, 3 and 4. There would be considerable benefits to the industry if the yields of these harvests could be significantly increased.

1.6 Dry matter yield components of pyrethrum

The flower dry matter yield per plant and per hectare is of economic importance in pyrethrum production as the flowers contain the final product of the crop. The pyrethrin yield of the pyrethrum crop is composed of two main yield components; flower DM yield and pyrethrin concentration or assay. The total flower DM yield weight is in turn made up of the number of flowers, weight of individual flowers, number of plants per unit area and number of flowering stems per plant (Parleviet 1970). The total pyrethrin yield of the crop is therefore determined by all of the components that determine the DM yield as well as the pyrethrin concentration. The lack of consistency in pyrethrin yield may be due to variability in the total DM yield and/or pyrethrin concentration, both of which may be influenced by environmental, genetic and management factors (Sastry and Sushil 2001).

Temperature is one of the main factors thought to affect flower yield and pyrethrin content. Flower initiation is promoted by the low temperature and the number of flowers initiated is low if plants receive insufficient chilling for vernalization (Brown and Menary 1994a; Glover *et al.* 1975). Analysis of commercial crop data from Tasmania has suggested that pyrethrin concentration also tends to be lower in seasons where temperatures during flower development are higher than average. Higher temperature also promotes more rapid flower maturation, and during the early stages of flowering may influence flower initiation (Brown and Menary 1994b) and

therefore flower number. Higher temperatures are likely to result in higher rates of photosynthesis and respiration, and may contribute to faster rates of flower development.

Differences in the patterns of photosynthate production and distribution within the plant under varying production environments and management practices may also contribute to variation in DM yield and assay by differentially affecting the rates of flower dry matter accumulation and pyrethrin accumulation. Changes in flower yield, rate of pyrethrin accumulation or duration of pyrethrin accumulation (stage of flower development at which pyrethrin accumulation ceases) could explain the seasonal variation in pyrethrin yield in Tasmania. A study in India reported plants exposed to low-minimum temperature produced a larger number of flowers with high dry matter content and total pyrethrins (Mohandass *et al.* 1986). While the requirement for a period of low temperature to induce flower initiation is well documented (Brown and Menary 1994a), none of the vernalization studies have determined effects of low and high temperature on flowering stem number under field conditions with fluctuating temperature. There is also little information on the effect of radiant energy on flowering yield and pyrethrin content including flowering stem initiation and stem number.

It is reported that the main factors affecting flower yield and dry matter content under east African production conditions were amount and distribution of rainfall and to lesser extent temperature, soil type, soil structure and fertility (Munro 1961; Parleviet 1970). Very high rainfall accompanied by persistent cloud cover was reported to reduce flower yield by 90% while shade could reduce yield by 50% (Muturi *et al.* 1969). Seasonal variation in cloud cover and rainfall are lower in the temperate region of Tasmania than the highland tropical regions of East Africa, but the scale of yield reductions reported suggests that differences in radiation levels and rainfall have the potential to contribute to yield variability in Tasmania.

Water stress at later FMS stages tends to reduce plant growth and flower DM. High rainfall and irrigation increased both the flower yield and pyrethrin content (Chung 1990; Muturi *et al.* 1969; Parlevliet 1970). Early research in Tasmania established the need for irrigation to ensure high crop yields. Irrigation at early flowering was reported to increase total dry weight by up to 32% and achene yield up to 20% when compared to non-irrigated plots. The increase was attributed to both increase in the

number of flowering stems/plant, the size of flowers and possibly the increase in number of flowers per plant (Chung *et al.* 1991). Irrigation in years one and two increased the pyrethrin content by up to 39%, which combined with the increase in the achene yield, gave an overall increase in pyrethrin yield of 64% (Chung *et al.* 1991). While not demonstrated in the literature, it is widely assumed in the industry that irrigation of pyrethrum crops results in slower rate of flower development and extended duration of pyrethrin accumulation. Pyrethrin assay may therefore reach higher levels in irrigated crops. Conversely, a period of water stress at the plant flowering stage has been shown to reduce the flower yield, number of flowering stems and DM weight of flowers (Brown 2005; Chung *et al.* 1991). There is little information on the levels of water stress on the crops that reduce yield and pyrethrin content or allow the plant to maintain growth and functions.

Variation in total pyrethrin and the flower DM yield may also be affected by flower maturity stages (FMI) at harvest. The pyrethrum inflorescence contains the majority of the plant's active compound approximately 1 - 2% pyrethrin (Casida and Quisad 1995). Over 90% of the total pyrethrin content was observed to be derived from achenes (disc florets) (Chandler 1951) and the accumulation of the pyrethrin in the achenes has been closely related with the development of oil glands in the flowers (Bhat and Menary 1979). The flower pyrethrin content increases with FMI stages but declines at higher FMI as rate of accumulation of DM is greater than accumulation of pyrethrin (Bhat and Menary 1979).

Additional sources of variation in pyrethrin yield are the management factors that influence total pyrethrin and flower yield. These include planting density, sowing times, application of nitrogen fertilizers and harvesting efficiency. Crops planted at high density of over 39 plants/m^2 produced higher flower DM yield than those planted at lower densities (Fulton *et al.* 2001). In Tasmania, seedlings need to be approximately 14 months old before they produce sufficient quantity of flowers for harvesting to be economical (Natural Resources and Environment, 1998). Sufficient vegetative growth after crop emergence is needed to ensure plants can support high flower yields. If planting time is delayed, resulting in limited vegetative growth prior to the winter period where growth is slow and vernalization occurs, substantially lower flower yields are obtained compared to earlier planted crops (Bhat 1995;

Fulton 1998). At harvesting if crops are left too long in the field to dry, degradation of pyrethrin is possible and yield reductions occur.

While a number of environmental factors and crop management practices have been shown to affect crop yield, and the components of yield have been defined for the crop, little is known of the effects of the various factors on individual yield components. Prediction of yield under field conditions, where a range of variables may affect different yield components, is therefore not possible. Identification of the key factors affecting yield under Tasmanian conditions, and understanding of the processes involved in yield formation, are required.

1.7 Project aims

In intensive and small scale pyrethrum production, consistent dry matter production is important to meet the supply contract. However, in intensive pyrethrum production in Tasmania, the flower dry matter yield varies significantly between growing seasons, between crops within seasons and is also variable across individual production sites. The pre-harvest sampling of DM yield and assay used by the industry to forecast harvest is only poorly correlated to the average yield per hectare obtained at commercial harvest (Brown 2005), highlighting the need for alternate yield prediction tools. This project aims to provide some of the information needed in the development of these tools.

A number of studies on the main cultural and environmental factors influencing pyrethrum production in the cool climate of Tasmania have been undertaken since introduction of the crop but most have not looked intensively into factors affecting the dry matter yield. Brown (2005) explained the variability in assay between crops in the 1999/2000 season was due to differences in both flower dry matter yield and pyrethrin content but did not further investigate the factors involved. A study on effects of irrigation on flower yield at the flowering showed improved total flower yield per hectare (Chung *et al.* 1991) but did not identify how irrigation influenced the yield components contributing to flower dry matter yield. Different levels of plant density had effects on flower yield and pyrethrin content (Fulton *et al.* 2001) but again the study did not identify the yield components contributing to differences in the flower dry matter yield.

Studies on major temperate crops have shown that the main factors affecting dry matter yield were light interception, nitrogen and water stress (Hay and Walker 1989). Differences between seasons and production locations in total radiation received by the crop throughout the growing season may explain a large part of the variation in crop DM yields. Pyrethrum is a perennial crop, unlike other temperate climate crops where relationships between DM yield and radiation interception have been demonstrated, and only a small percentage of the plants DM is partitioned to the flowers. These differences, and the accumulation of the secondary metabolite pyrethrins as a component of crop yield, add complexity to the processes affecting

yield. The project aims to determine if radiation interception explains a significant proportion of yield variability in pyrethrum crops.

The relationship between light interception and DM yield may be masked by effects of factors such as low temperatures, frosts, flooding, water stress or leaf disease. Water stress may reduce the leaf area for radiation interception, conversion of light energy to DM and partitioning of plant DM. Water stress has been identified as an important factor limiting dry matter yield in pyrethrum, so the project aims to analyse the effects of water stress on yield development. Plant density has also been noted to affect yield in pyrethrum, and an analysis of the effects of density on yield components will be undertaken to provide greater depth of understanding of the processes determining yield in the crop.

Chapter 2: The effect of light interception on flower dry matter yield of pyrethrum

2.1 Introduction

The interception of solar radiation by crops and use of the radiant energy to produce biomass and therefore dry matter yield is fundamental in crop production. Crops grow when they intercept light and carryout photosynthesis, and accumulate DM which may be partitioned to plant parts for vegetative and reproductive growth. The larger the leaf area the crop has, the higher the light interception, rate of photosynthesis and DM yield of the crop. Many researchers have found a linear relationship between photosynthetic active radiation (PAR) intercepted by canopy and crop growth rate (CGR) in spring wheat (Saini and Nanda 1986; Takahashi and Nakaseko 1993), reaching an asymptote in the relationship at the irradiance corresponding to light saturation (Field 1983).

The interception of light over the growing season by a crop can be used to explain the DM yield of crops. In some crops, the DM yield can be explained by canopy development or the levels of light available over the season. However, this may not be the case for accumulation of flower DM yield of pyrethrum crops. Pyrethrum is a perennial crop and in a temperate climate grows throughout the year, but flower development occurs over only part of the year. Light intercepted in this short period may be more important in flower DM yield than light interception over the entire season.

The total radiation required from the DM production in crops comes from the sun. The light intercepted by green leaf area for fixation of carbon during photosynthesis is known as photosynthetic active radiation (PAR). This radiation has the wavelength range from 400-700 nm and is measured as photosynthetic photon flux density units (PPFD) (Papadopoulos and Pararajasingham 1997). The PAR interception at crop level is related to the proportion of the crop area that is covered by the canopy and the degree of overlap between leaves in the canopy or leaf area index.

The crop canopy in field trials may be estimated by visual assessment, assessed directly by manual measurements of individual leaf areas or indirectly by taking

photographs and analysing the image to determine the canopy size (Purcell 2000). The indirect measurements of light interception are suitable for perennial crops such as pyrethrum as they do not disturb the crop or destroy the plant (Purcell 2000). Canopy size estimation using this method has not been reported for the pyrethrum crop, but could be used in assessing the relationship between canopy coverage, light interception and DM yield.

The complete interception of incoming PAR may be achieved in crops where sufficient overlap between leaves occurs. With large thick, undissected and horizontal leaves, this occurs with a leaf area index (LAI) value close to 1. Crop species display a wide range of leaf sizes, shapes, thickness and dispositions but a LAI of 3-5 is generally required for interception of more than 90% of incoming energy in a range of species and varieties (Rawson *et al.* 1984). For maximum interception of light and crop dry matter production in a cool temperate climate, the peak leaf area index of crops should coincide with the timing in the season of maximum peak radiation (Hay and Porter 2006; Hay and Walker 1989). However, where crop components such as flowers are to be harvested, management practices used to achieve peak LAI at peak radiation may not be available. Little information on LAI of pyrethrum crops in temperate climate exists.

The main factors that limit efficient interception of light and affect the rate of photosynthesis are variation in radiation and canopy size (Hay and Porter 2006; Hay and Walker 1989). Fluctuation of radiation is one of the main factors affecting efficient interception of light for maximum photosynthesis. In a cool temperate climate there is fluctuation in total radiation on an hourly, daily basis, and monthly because of seasonal changes in day length. The general pattern of availability of total radiation annually in a cool temperate climate is a broad peak in mid-summer and lowest levels in winter (Purcell 2000). Other microclimatic factors such as a cloud cover, rainfall and topography add variation to the availability of total radiation at different sites and can affect amount of radiation intercepted.

Variation in canopy size is another factor limiting efficient interception of light. In most temperate agricultural crops, the peak leaf area index does not coincide with maximum radiation (Hay and Porter 2006; Hay and Walker 1989). In annual crops like cabbages, broccoli, potato and wheat the life cycle of the crop is short and the potential to increase light interception outside the period of peak leaf area is limited

by low temperature, environmental hazards such as frost, flooding, drought or leaf diseases and the need for the crop to be mature at a time when soil physical conditions permit the necessary harvest operations (Hay and Porter 2006; Hay and Walker 1989). In perennial crops species like pyrethrum the pattern of interception is different as the crop has long life cycle. Under appropriate cutting or grazing management a continuous canopy maintained throughout the year resulting in high interception of high proportion of available PAR may be possible (Hay and Porter 2006) .

The leaf nitrogen status, water stress and plant density are the main factors affecting the canopy size. Lack of nitrogen limits vegetative growth and decreases leaf area and increased supply of nitrogen increases leaf size, stimulates branching, enhances survival of branches and improves photosynthetic capacity and in particular variation in leaf carbon dioxide exchange rate (Evans 1993; Hay and Porter 2006). The nitrogen application and management program for pyrethrum production in Tasmania reduces the risk of restricted canopy development due to nitrogen deficiency.

Water stress during canopy development tends to reduce the leaf area of crops and hence light interception. However, timely rainfall and irrigation can lead to some degree of recovery of the canopy following water stress, improving interception of light. Pyrethrum is grown as an irrigated crop in Tasmania and the irrigation program has supported increased yield of the crop compared to the previous dryland production system.

The high canopy or leaf area per unit area is important to intercept maximum radiation. The canopy size per unit area is determined by the number of plants per unit area and their leaf area index. At low crop density, the canopy area or canopy density is low and hence low interception of light occurs, while at high crop density greater interception of light is possible. Fulton (2001) reported high density of up to 39 plants/m^2 provided maximum yield. However the study reported only pyrethrin yield and did not examine light interception and DM production. The other important factors to influencing canopy size are plant differences in crop species in leaf size, pest and diseases and changes in local microclimate (Hay and Porter 2006; Hay and Walker 1989).

The research into pyrethrum production in Tasmania has considered some aspects of light interception (Brown and Menary 1994a; Chung 1990; Fulton *et al.* 2001; Groom 2003). Crop management has been planned so that all sowing and harvesting activities are carried-out according to seasons and the crop canopy reaches maximum LAI to coincide with peak radiation in spring and summer (Bhat and Menary 1984b; Faber 1980; Macatta 2001). Crop density has been increased from 5 to 39 plants/m^2 and nitrogen fertilizer application rate and timing optimised to enable efficient interception of light (Chung *et al.* 1991; Fulton *et al.* 2001). Breeding of improved clones with high yield and pyrethrin content as well as flower synchronicity has also been undertaken (Bhat and Menary 1984b; Groom 2003). However, despite the appropriate management applied, the DM yield of pyrethrum in Tasmania remains variable between crops and season. The visual observation of pyrethrum crops in field conditions has indicated green leaf area of crops varies between plots within crops, between crops, and between seasons, indicating variability in light intercepted may occur. However, there is no available scientific information specifically on light interception by the pyrethrum crops and relationships between light interception and the flower biomass yield and pyrethrin content. The aim of this study was to determine how light interception influences crop growth, canopy development and partitioning of dry matter to the flowers of pyrethrum.

2.2. Materials and methods

2.2.1 Field trial design

The crop grown at the sites used in the data collection were produced for BRA and owned by the contracted farmers. The eight crops used in 2006 were designated according to the BRA crop record system 45501, 50110, 55904, 55803, 63211, 64205, 72504 and 75301. In 2007 crops were 45501, 50907, 53506, 53506, 59504, 62304, 64206, and 74401. The crops were spread-out in the inland of northwest Tasmania and covered a range of microclimates, soil types, moisture levels and topography.

Pyper was the main commercial pyrethrum variety grown in all sites and the crops were a mixture of first and second season crops of varying areas. The management of the crops was done by BRA up to harvest.

2.2.2 Climatic conditions

The average daily total radiation data as well as other weather data were recorded daily during the pyrethrum growing season at the Forthside Vegetable Research Centre in northwest Tasmania. All crops were located within 100 km of the weather station.

2.2.3 Canopy size estimation

The ground coverage or canopy size was estimated indirectly by taking photographs of the crop from early spring to mid-summer, August to November in 2006 and April to December in 2007. Within a crop, 20 plots in different parts of the field were marked and digital photographs taken every 7 days until the flower crown reached full size and completely covered the plant surface. The camera was mounted on a stand at fixed height above the soil surface. The digital photographs were then downloaded into a computer and the images analysed using Fovea Pro 4.0 (Reindeer

Graphics Inc.) software in Adobe Photoshop CS2 (Adobe Systems Inc.) to determine the size of the green leaf area of the crop.

2.2.4 Validation of image analysis procedure

The accuracy and precision of the image analysis routine were validated using 10 original (unprocessed) images randomly selected from the 2006-07 season. The image estimates were then corrected by overlaying the black pixels of the threshold image over the original image and then removing or adding pixels in the threshold layer to match that of the canopy cover of the original image. The original images were assessed a further two times for percentage ground cover. The error in the estimate of percentage canopy cover was then calculated by subtracting the corrected cover from that computed from the original image. The images produced during this study were processed in bulk using the Adobe Photoshop batching routine. The accuracy of the batch processing was cross checked by overlaying the threshold layer over the original image, and converting the black pixels to a transparent red. This blended image was then saved for visual cross checking. Under estimates of foliage appeared as green foliage, while overestimates were revealed as extraneous red features. Gross computer errors in processing were identified using this technique and affected images were reprocessed and again visually crosschecked.

2.2.5 Measurement of yield

At commercial flower maturity plants were harvested and plant samples partitioned into leaf, stem and flowers. These were then weighed before and after drying at 70˚C for 48 hours. A subsample of flowers was taken and sorted into the FMI stages. The pyrethrin content of the crop was analysed by the BRA Laboratory and pyrethrin yield per plant was calculated along with yield on a per unit area (kg/ha) basis.

2.2.6 Analysis of data

The cumulative solar radiation intercepted by the crop during the growing season in 2006 and 2007 were estimated using canopy area and volume. The solar radiation

intercepted by the canopy was estimated by multiplying cumulative total daily radiation from Forthside Vegetable Research Centre data with % ground cover. The data were then analysed using the general linear model procedure in SAS (SAS 1990) for comparison of means. Fishers (Steel and Torrie 1980) least significant difference (LSD) was calculated at $P<0.05$ level of probability unless otherwise specified. Correlations were analysed using the correlation procedure in SAS.

2.3 Results

2.3.1 Climate and total radiation for the 2006-08 seasons

The pattern of daily total radiation received by the crops varied between the 2006, 2007 and 2008 cropping seasons (Figure 2A, B & C). In all years, the total radiation was lowest from July to August and then increased reaching a maximum of 30 MJ/m^2 in December. There was considerable daily variation in radiation in all three years. The pattern showed seasonal effects on availability of radiation with low in winter and increasing to moderate in early autumn and reaching maximum peak in mid- summer. The peak period of total radiation was between the months of late November and mid December.

The mean monthly rainfall for years 2006, 2007 and 2008 (Table 2.1) were very low, below 6 mm per month. The high rainfall was from May to September while years with the highest total rainfall were 2007 and 2008. The mean monthly minimum and maximum temperatures were high from September to April while low from May to August (Table 2.1). The variation with mean monthly rainfall, minimum and maximum temperature was due to seasonal effects.

Table 2.1 The mean monthly rainfall, minimum and maximum temperature at Forthside Vegetable Research Centre from January to December in 2006, 2007 and 2008.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Rainfall (mm)													
2006	27	6	24	109	50	33	79	25	54	13	13	24	458
2007	74	17	42	21	194	27	76	123	86	40	24	124	846
2008	13		34	62	76	86	128	67	77	9	88	58	698
Temperature													
Minimum (˚C)													
2006	12	11	11	7	5	3	5	5	5	6	8	9	
2007	13	15	11	10	9	3	4	6	6	7	10	12	
2008	13	12	11	9	8	7	4	4	6	8	9	9	
Maximum (˚C)													
2006	21	21	20	15	13	12	12	13	14	15	18	19	
2007	22	24	11	19	16	12	12	13	14	15	20	20	
2008	23	21	21	17	15	14	12	13	14	17	18	19	

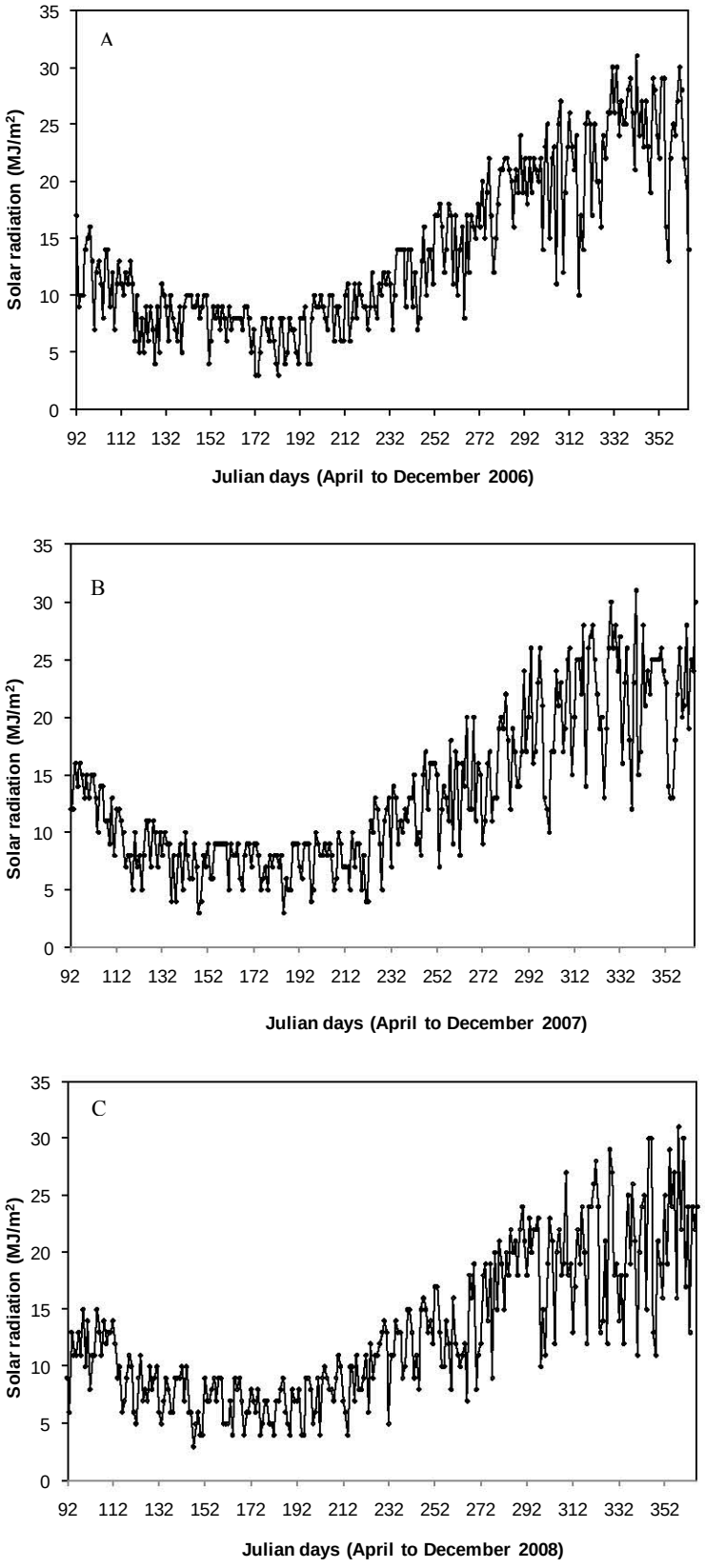


Figure 2.1 The daily total solar radiation from April to December in A) 2006, B) 2007 and C) 2008.

2.3.2 Light interception across and within sites in 2006

Ground cover was calculated and presented as green leaf area expressed as a % of the total plot area over time (Figure 2.2). The mean ground cover of the crops increased over the season, reached peak in spring and then declined as the flower white petals began to cover the leaves (Figure 2.2). The % ground cover increased from a low of 40% in April and reached a maximum of 80% in late October to November (Figure 2.2). Ground cover accumulation varied among (Figure 2.2) and within (Figure 2.3) crops, but good crops with large initial canopy size and interception of light tended to maintain a large canopy throughout the growth period reaching a broad peak with early flower maturation. The outlier crop (55904) was well below average ground cover indicating a poor crop with delayed development and flower maturity (Figure 2.2).

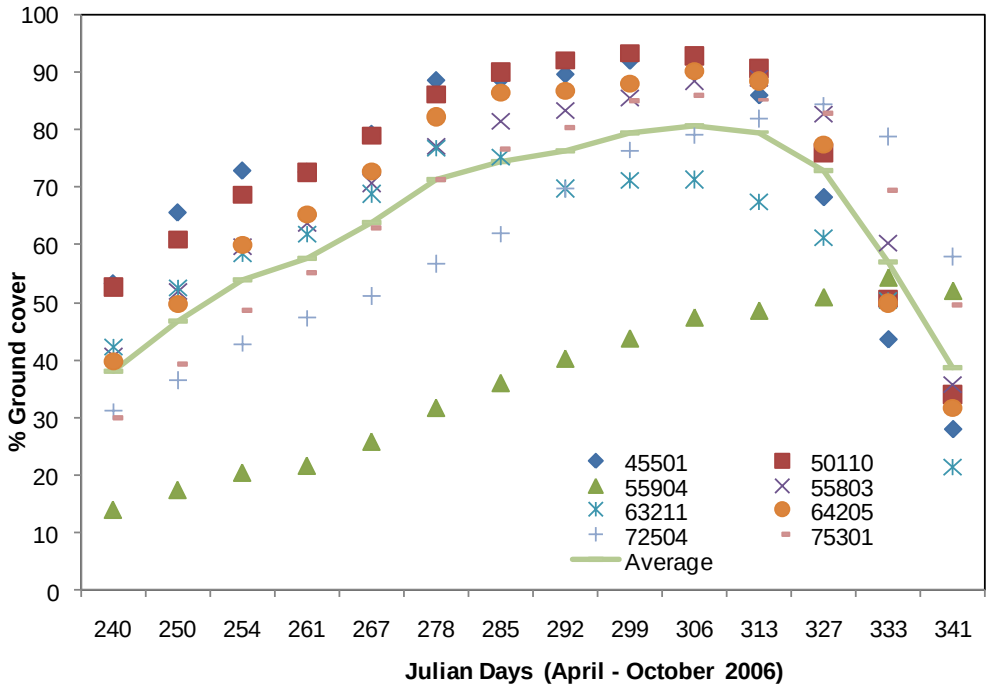


Figure 2.2 The line in the graph shows the mean pattern of % ground cover of eight pyrethrum crops from April to October in 2006. The coloured markers were the pattern of % ground cover of the eight pyrethrum crops in the same period

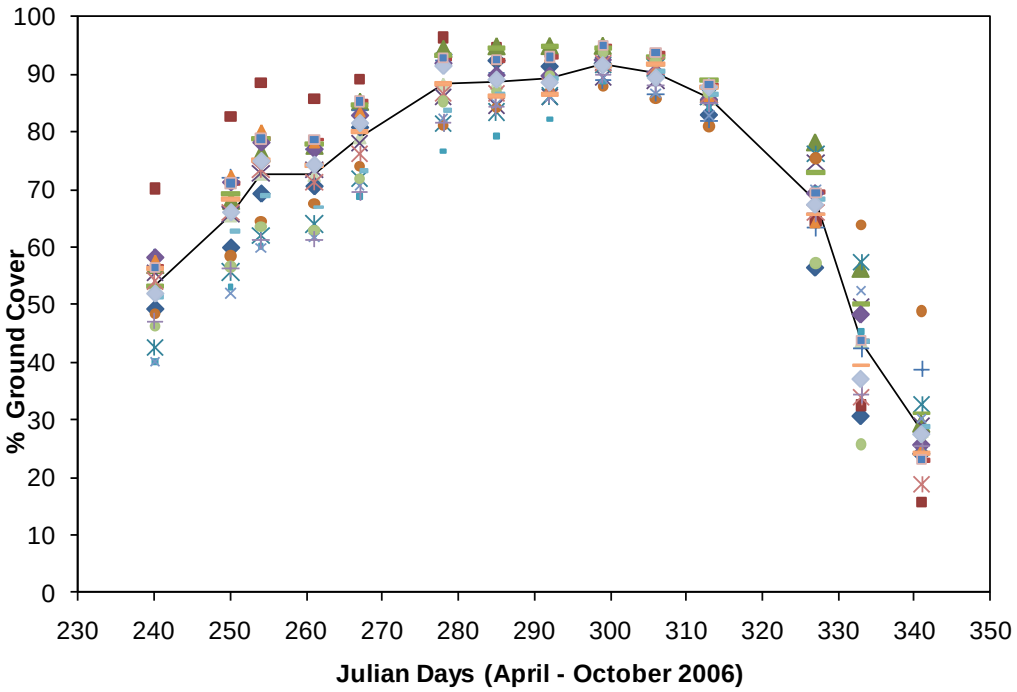


Figure 2.3 The line shows the mean % ground cover of 20 plots of pyrethrum crop within single pyrethrum crop (44501) from April to October in 2006 growing season. The coloured markers show the variation in individual green leaf cover of the 20 plots.

2.3.3 Ground cover and biomass yield of crops in the 2006 and 2007 growing seasons

Variation in light interception was hypothesised to contribute to differences in pyrethrum yield. This was examined by calculating the cumulative light interception from August to October by multiplying % ground cover by radiation for crops in 2006 and 2007. The analysis however showed that cumulative light interception varied among crops and across years and had an inconsistent relationship with components of yield (Table 2.2 and Table 2.3).

Generally, cumulative light interception (CLI) was greater in 2006 than in 2007, while flower yield was smaller. There was a strong positive correlation between CLI and all yield components across crops in 2006 (Table 2.2) but not in 2007 (Table 2.3).

Table 2.2 Average light interception reported as cumulative radiation multiplied by canopy area and yield of eight pyrethrum crops between April and October in 2006. The lsd is at P=0.05.

Crop	Canopy area x radiation (MJ/m^2)	Flower DM (g/m^2)	Pyrethrin Yield (kg/ha)	Flower Assay (%)	FMI	Flower density (flowers/ m^2)
45501	110410	382	69	1.95	479	2280
50110	116475	304	70	2.52	451	1751
55904	69839	52	7	1.35	332	160
55803	114336	358	62	1.90	543	2080
63211	88871	207	30	1.60	416	1509
64205	112430	284	49	1.88	528	1774
72504	109663	270	46	1.80	486	1497
75301	115676	270	46	1.80	486	1497
Average	104712	265	47	1.84	465	1568
lsd	5521	39	8	0.10	31	208
Correlation	-	0.94***	0.94***	0.81*	0.85**	0.88**

*,**,*** indicates statistical significance at P = 0.05, 0.01 and 0.001, respectively

Table 2.3 Average light interception reported as cumulative radiation multiplied by canopy area between April and October and yield of nine pyrethrum crops in 2007. The lsd is at P=0.05.

Crop	Canopy area x radiation (MJ/m^2)	Flower DM (g/m^2)	Pyrethrin Yield (kg/ha)	Flower Assay (%)	FMI	Flower density (flowers/ m^2)
45501	11557	500	77	1.66	544	1550
50907	95774	368	51	1.49	454	1517
53006	94789	506	76	1.63	600	1666
53506	71444	314	39	1.33	594	969
59504	71512	386	62	1.70	516	1581
62304	19213	362	54	1.64	449	1905
64206	21478	463	72	1.69	507	2229
72504	21478	166	25	1.70	547	465
74401	88539	381	65	1.84	437	1745
average	76241	376	60	1.60	516	1514
lsd	7124	61	9	0.17	50	232
Correlation	-	0.11	0.07	-0.02	-0.09	-0.04

*,**,*** indicates statistical significance at P = 0.05, 0.01 and 0.001, respectively

2.3.4 Light interception and biomass yield within crops in the 2006 and 2007 growing seasons

In section 2.3.2 the results showed that the relationship between light interception, measured as cumulative radiation intercepted, and components of yield were inconsistent across years. More detailed analysis of the data shows that there was a high level of temporal and spatial variation in light interception across and within sites.

Data for yield components of flower DM and pyrethrin yield were plotted against cumulative radiation x canopy area in 2006 and 2007 (Figures 2.4 and 2.5). In 2006, both flower DM and pyrethrin yield tended to have a positive relationship with cumulative radiation intercepted but there is a large differences in variation among crops. In 2007, there is a similar degree of variation among crops, but some crops (64206 and 72504) were outliers, with high yields apparently associated with low cumulative radiation intercepted (Figure 2.5).

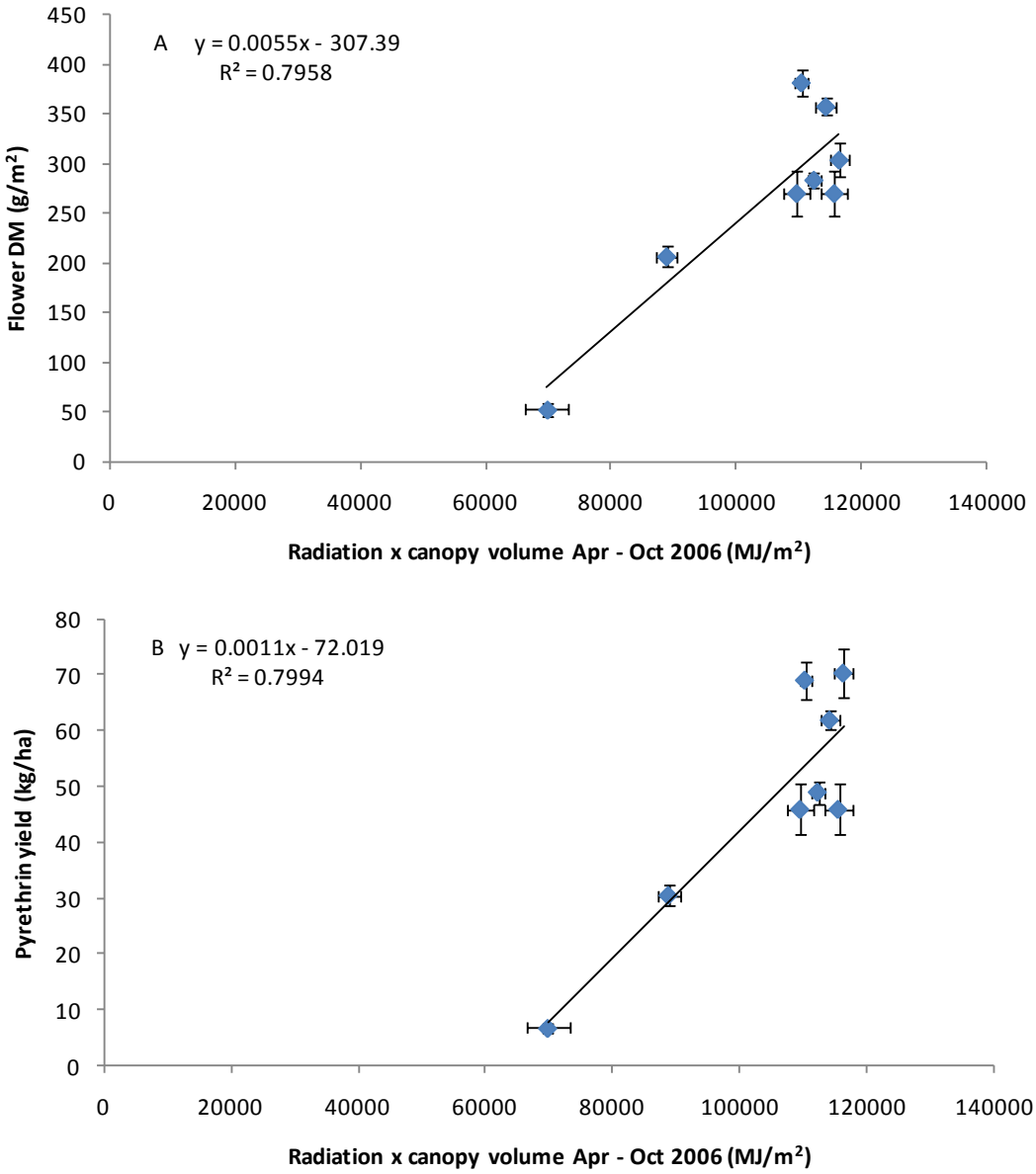


Figure 2.4 The relationship between average (A) flower DM yield and (B) pyrethrin yield against cumulative radiation intercepted by canopy area across 9 crops for the Aug-Oct 2006 growing season. Bars represent the SE.

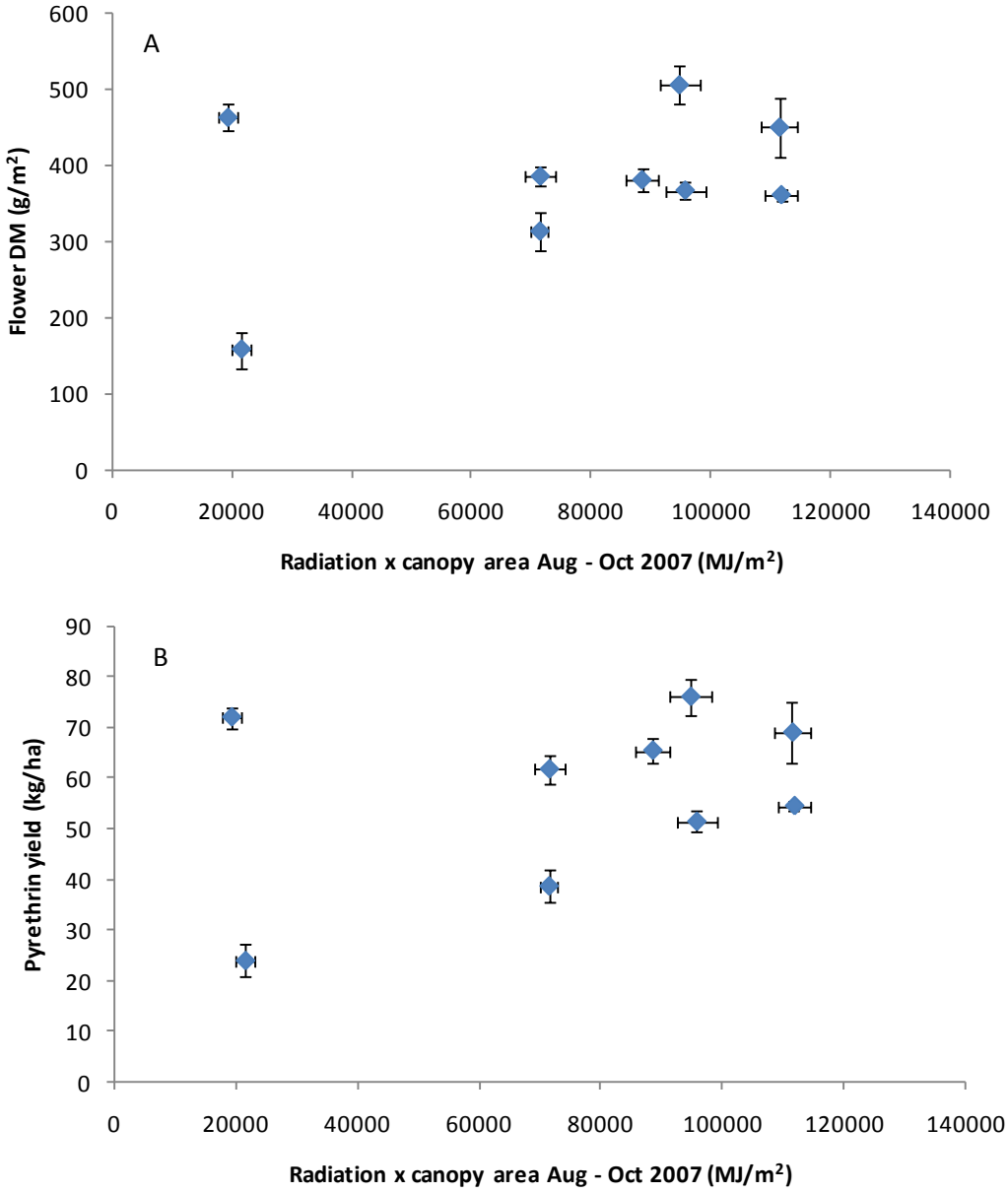


Figure 2.5 The relationship between average (A) Flower DM yield and (B) pyrethrin yield against cumulative radiation intercepted by canopy area across 9 crops from Aug-Oct 2007 growing season. Bars represent the SE.

2.3.5 Relationship between light interception and yield in 2008

The data for canopy area, flower DM yield, pyrethrin yield, flower biomass, stem density and flowers per stem for eight crops in 2008 are presented (Table 2.4). Canopy area was assessed at an approximate FMI of 200-300 as an indication of radiation intercepted by the crops during the growing season (Table 2.4). There was a moderate positive relationship between the flower DM yield, flower biomass, stem density and flowers per stem against canopy area among crops (n=8) in 2008. There was also a large variation in stem density between the crops, ranging from as low as 75 stems/m^2 to as much as 600 stems/m^2 (Figure 2.4). There was comparatively large variation in flower DM which ranged from 90 to 278 g/m^2 and this variation was attributed to the maturity at which flowers were harvested (FMI). This effect can be seen in a comparison of the data for crop 64206 and 53006 (Table 2.4).

The analysis among crops showed correlation was highly significant ($P<0.001$) between flower DM, stem density, flower density, flower DM and flowers per stem against canopy area (n=8) in the month of October (Table 2.4).

The data for flower DM, pyrethrin yield and flower biomass were plotted against canopy area for eight crops in October 2008 (Figure 2.7A, B and C). The flower DM, pyrethrin yield and flower biomass yield tended to have positive relationship with canopy area but there was large differences the variability of flower DM yield, pyrethrin yield and flower biomass yield between the crops (Figure 2.7A , B and C).

Table 2.4 Effect of crop on % canopy area in October and partitioning of DM in 2008. The lsd is at P=0.05. Correlations are among crops, n=8.

Crops	Canopy area Oct (%)	Flower DM (g/m^2)	Pyrethrin yield (kg/ha)	Stem density ($stems/m^2$)	Flower density ($flowers/m^2$)	Flowers per stem
45501	76	251	56	321	1785	5.4
50907	85	204	43	296	1560	5.1
53006	69	278	64	249	1465	6.0
53506	54	217	48	205	1181	6.1
59504	76	194	43	312	1652	5.6
62304	94	261	6	607	2001	3.4
64206	31	90	18	85	523	6.1
74401	69	203	38	352	1659	4.9
Average	69	212	40	303	1478	5.3
Lsd	7	50	10	75	250	1.0
Correlation		0.73*	0.01	0.85**	0.94***	-0.73*

*,**,*** indicates statistical significance at $P = 0.05$, 0.01 and 0.001, respectively

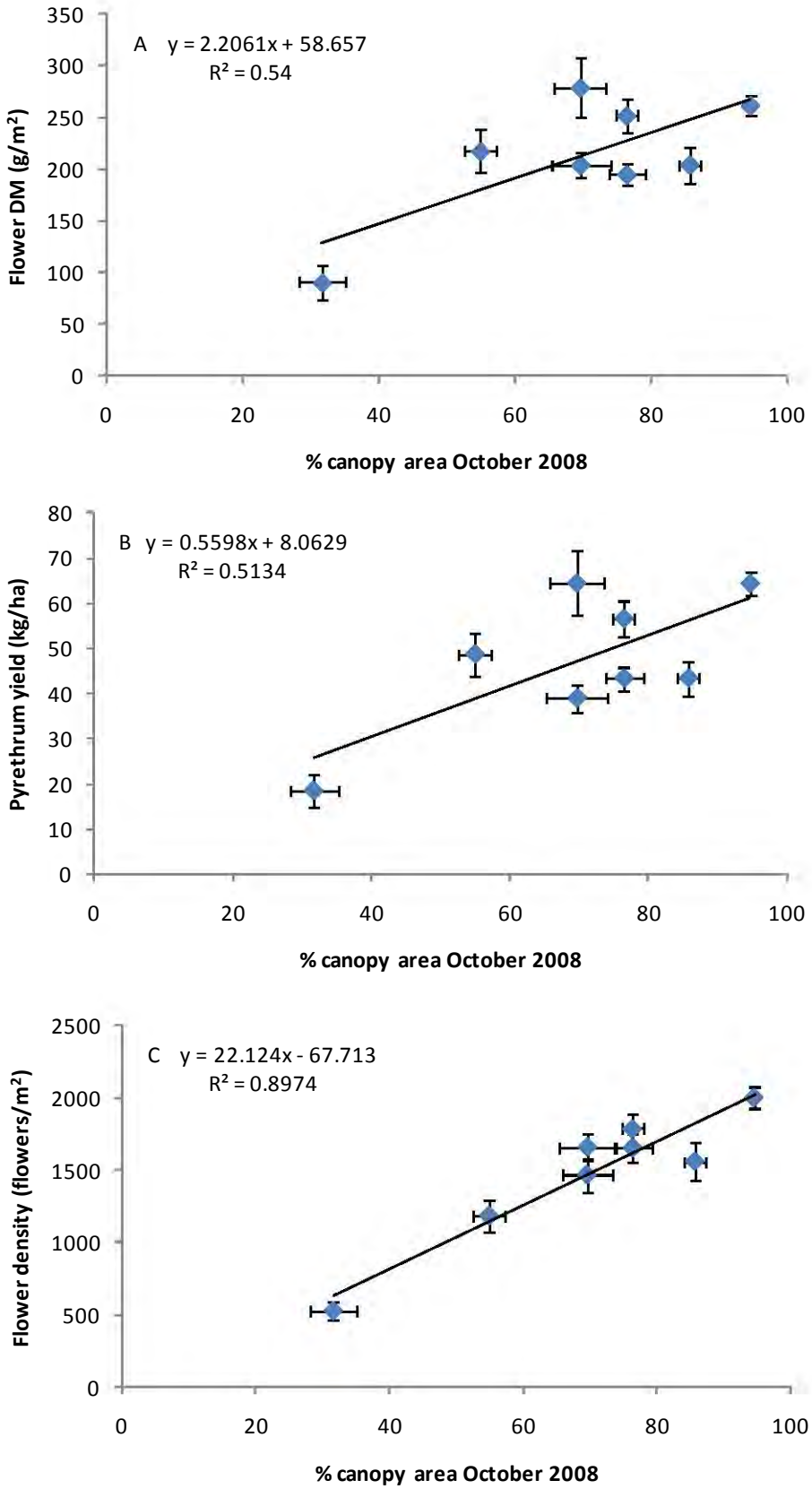


Figure 2.7 Plot of canopy area against (A), flower biomass (B), pyrethrin yield (C) flower density within eight crops in October 2008 growing season.

2.4 Discussion

The aim of the study was to determine if there was correlation between light intercepted and flower DM yield and pyrethrin content. The analysis showed a positive correlation with flower DM yield, pyrethrin yield and flower biomass in 2006 and but no correlation in 2007, indicating seasonal and site effects. However there was a great variability in canopy area, radiation intercepted and the crop yield across and among crops in 2006 and 2007. In the 2008 season there was significant correlation between flower biomass, pyrethrin yield, stem density, flower density and flowers per stem against canopy area across and among crops. The moderate and sometimes strong correlations suggested significant site effects such as variation in canopy coverage, canopy density and water stress.

Variation was evident in the mean total daily radiation throughout the season, year during period of crops growth (Figures 2.1, 2.2 and 2.3). The variation in radiation maybe explained as due to seasonal changes in day length and daily weather pattern that had influence on daily total radiation. The period of maximum and peak radiation occurred in summer from November to December. The similar pattern of leaf area growth (Figure 2.2) suggested seasonal effects on total radiation intercepted by crop canopy during the period. The pattern was typical of annual temperate crops like sugar beet, wheat, potato, barley and oat crops studied (Hay and Porter 2006).

Variation was also observed in % ground cover within and among crops in 2006 (Figure 2.1). This suggests variation in light intercepted by crop canopy depended on availability of total radiation throughout the growth season and site effects. The peak LAI of the crop (Figure 2.2) tended to coincide with the peak total radiation (Figure 2.1) suggesting the possibility of maximum interception of light by the crops. The variation in light intercepted was due to seasonal effects with variation in total radiation throughout the year due to changes in day length caused by the seasons and site effects mainly with variation in crop canopy density. Other potential sources of variation among sites were likely to have been related to the management of inputs such as fertiliser and irrigation timing and quantities. For example, variation in crop canopy size has been linked with water stress levels within sites (Fulton *et al.* 2001; Groom 2003).

There was no significant correlation between the flower DM yield, pyrethrin yield and flower density in 2007 season (Table 2.3). The differences in crop yield with high average in 2006 while low in 2007 as shown by crop 45501 and 72504. This can be explained by the seasonal effect with variation in total radiation and rainfall in 2006 and 2007 (Figure 2.1, Table 2.1) and site effects with variation in leaf area and radiation intercepted within crops in 2006 compared to 2007.

The variation in flower DM and pyrethrin yield was observed in 156 plots across crops. This may be explained in two general hypotheses. Firstly, the partitioning of accumulated DM in leaf tissue during photosynthesis to other vegetative and reproductive parts like stem and flowers may explain this. The carbon accumulated may not be partitioned constantly to flowering stem for flower development and production. This suggests other plant parts within the same spot within sites were interfering with the interception of the same light for photosynthesis and the proportion of photosynthate partitioned to flowers was unequal. Secondly, the other factors likely to affect allocation of DM to stem leaves and flowers were the genetic differences between the plants or environmental stress like moisture stress. When this happens, the plant respond by partitioning the DM to other vegetative parts of the plants like stem, roots. The typical response of plants to stress especially under drought stress during vegetative growth is reducing its photosynthetic activity, and increases assimilate partitioning to roots in favour of shoots. These strategies change under water stress during reproductive growth, and assimilate are preferentially allocated to the flowers. During this time, due to competition for assimilates between roots that are trying to find more moisture, and the flowers producing fruit, the plant becomes more sensitive to moisture stress (Tiaz and Zeiger 2002). If pyrethrum behaves in a similar manner, environmental stresses during flower production could easily influence the level of flower biomass produced.

The other explanation of this could be the efficiency of conversion of intercepted radiation by the crop canopy into DM, and accumulation and variability in production of secondary metabolites such as pyrethrins between crops. The main factors influencing this could again be genetics or external factors such as drought stress, pest and disease which induce physiological stress within the plants, which in turn alters gene expression, photosynthetic capacity and the production of secondary metabolites such as pyrethrins. When this happens it reduces the

efficiency of conversion of intercepted radiation and could result in proportionally less radiation being converted to photosynthetic products such as flower DM.

The stem density per unit area had a significant effect on flower DM and pyrethrin yield and green leaf area (Table 2.4). The stem density influenced the flower DM and pyrethrin yield in two ways. Firstly, through number of stem an individual plant had and the number of flowers per stem. The high stem density had high flower number and high flower DM and pyrethrin yield as opposite to the low stem density. Secondly, the green leaf area or % ground cover increasing coincidently with stem number. Stem number itself is a combination of plant density and crown size. From this it is proposed that that yield and variability of that yield when measured as flower biomass, is fundamentally a function of plant stem density, and the number of stems per plant. Secondary to this is flower number per stem, for which the plant produces more at a low stem number, and decreases as stem number increases. At low densities however, flower biomass is likely to be more variable due to the increased variability in flower number. Underlying this model of dry matter allocation to flowering is the synthesis of pyrethrin, which is also likely to vary according to physiological, genetic, environmental variables that influence this production of these metabolites. This understanding of the crop can be able to used predict dry matter allocation and maybe also predicting pyrethrum yields at harvest.

Significant variability in crop biomass yield within and among crops suggested differences in canopy coverage, canopy density and light interception due to seasonal and site effects. The differences in canopy density and light intercepted also resulted in difference in rate of conversion of light to DM yield, accumulation in plant tissue and partitioning to crops vegetative and flower DM and pyrethrin accumulation among the crops. The moderate positive relationships identified suggested that seasonal and site effects significantly influenced this relationship, and one of the main factor was stem density and variation in canopy density. It was hypothesised that differences could be related to variation in plant density and drought stress, which can occur during the later stages of flower development in Tasmania.

Chapter 3. The effect of plant density on dry matter and pyrethrin content of pyrethrum

3.1 Introduction

The weak correlation between light intercepted and DM yield in pyrethrum, both within and between crops described in Chapter 3, might be explained by variation in plant density. The experiments described in this chapter evaluated how variation in plant density affected DM partitioning and pyrethrin content.

The effect of competition between plants within an environment or population has been extensively studied (Botwright *et al.* 1998; Chung 1990; Fulton *et al.* 2001; Holliday 1960). Competition can be defined as the use of a resource by one individual, which reduces the availability of that resource to other individuals, whether of the same species (intraspecific competition) or other species (interspecific competition) (Rickletts 1990). Environmental factors of light, water and nutrients are well-known drivers of interplant competition (Baldwin 1976; Holliday 1960; Willey and Heath 1969).

At establishment, plants are widely separated and rarely interfere with each other, but as growth proceeds and demand for resources increases, each plant will modify the environment of adjacent plants, resulting in competition (Donald 1961; Milthorpe and Moorby 1979). The stage of development at which competition commences and the degree of competition is influenced by plant density, species under investigation, rate of growth and plant spatial arrangement (Grey *et al.* 1991). In general, competition between individual plants will occur at an earlier growth stage at a higher plant density. This is because individual plants are competing for smaller shares of water, nutrients and light. Growth will therefore be reduced and plants will be of lower DM weight than at lower densities. However, individual plant size at high densities may be compensated by the increased total biomass of the crop, which is usually at least equivalent to that of plants at lower densities as there are more plants (Harper 1977; Papadopoulos and Pararajasingham 1997). The relationship between total crop biomass and plant density is usually asymptotic, approaching a constant biomass at high densities (Holliday 1960). In contrast, the relationship

between yield of fruit or seed is typically parabolic, where yield increases to a maximum and then decreases with increasing plant density (Holliday 1960).

Intensive modern agriculture aims to minimise water and nutrient deficits so that competition for light then dominates plant growth (Frappell 1979). Competition for light occurs in the early stages of growth before canopy closure and therefore smaller plants are not able to get enough light to sustain growth (Aikman and Benzamin 1994; Donald 1961). This results in an uneven distribution and interception of available light among individual plants and leads to alteration of size structure of the population and self-thinning (Weiner and Thomas 1986; Yoda *et al.* 1963). The greatest self-thinning and mortality occurs at the period of rapid growth when there can be intense competition among individual plants (Antonovics and Levin 1980; Yoda *et al.* 1963).

Planting densities used to establish pyrethrum in tropical regions have traditionally been very low, at approximately 4 plants/m^2 (Parlevliet *et al.* 1968). A study in Kenya in 1966 reported yield increase in response to higher planting densities, but the advantages were not considered economical due to the high cost of manual establishment (Parleviet and Brewer 1971). An optimum yield was reported to be obtained from 5.5 plants/m^2 (Rao and Singh 1982) and 4.4 plants/m^2 in India (Sastry *et al.* 2001; Sastry *et al.* 1989).

A planting density of 4 plants/m^2 was initially used when pyrethrum was introduced as a new crop in Tasmania, even though the temperate climate was conducive to only one cycle of flower production per year. Flower yields in Tasmania from pyrethrum crops established from vegetative divisions or splits at planting densities of 2 - 4 plants/m^2 were between 1700 to 2400 kg/ha (MacDonald 1995; Salardini *et al.* 1994). Research undertaken in Tasmania in the late 1990's using direct-seeded pyrethrum however suggested that the optimum planting density for pyrethrum was much higher, at 16 plants/m^2, which produced a dry flower yield of 4500 kg/ha (Fulton *et al.* 2001). Densities greater than 40 plants/m^2 were reported to result in self-thinning due to interplant competition. The increase in flower yield was apparently associated with more tillers per plant, although flowers per tiller was suggested to regulate flower DM yield at low planting densities of less than 8 plants/m^2 (Fulton *et al.* 2001). Pyrethrin concentration remained constant at around 2.30% and hence pyrethrin yield was directly related to flower DM yield. Statistical evidence in

support of the results on the effect of planting density on flower and stem number was however not clearly presented. Furthermore, although this was a replicated field trial, planting density treatments were imposed by changing within row spacing, with between rows set at either 300 mm at low densities or 150 mm at higher densities (Fulton *et al.* 2001). Thus, rectangularity varied among the plant density treatments, which is likely to have influenced the timing and intensity of interplant competition, particularly within rows. Growers have implemented these recommendations and now use a planting density of around 25 plants/m^2 (Fulton *et al.* 2001; Natural Resources Environment 1998), although with equidistant plant spacing of 35 cm and recently released varieties.

Variation in plant architecture, crop biomass, flower yield, pyrethrin content and pattern of pyrethrin accumulation has been reported between varieties (Faber 1980; Ikahu and Ngugi 1988; Ikahu *et al.* 1994; Parlevliet *et al.* 1979). It is unknown whether these differences in response between varieties affect the optimum planting density to maximise crop growth, yield and pyrethrin content of pyrethrum. Thus there is a need for a detailed evaluation of the impact of planting density and family, using equidistant plant spacing, on the partitioning of DM between plant components and pyrethrin content of pyrethrum.

3.2 Materials and methods

3.2.1 Genetic variation in dry matter partitioning in response to plant density in pyrethrum

The response of 19 selected families of pyrethrum to three plant densities was examined in a randomized complete block design with four replicates. The three plant densities were low (40 x 40 cm; 16 plants/m^2), medium (35 x 35 cm; 25 plants/m^2) and high (25 x 25 cm; 44 plants/m^2). The trial was conducted at a research farm (41°10'S, 146°40'E) in northwest Tasmania in the 2008-09 growing seasons. The trial was harvested when plants reached commercial maturity. Whole plants were labeled, tied and cut at ground level and brought to the laboratory for processing. The number of primary flowering stems was counted and plants were stripped of flowers and the fresh weight of flowers recorded. A sub-sample of approximately 200 g of flowers were taken from each plant and dried at 50^{o}C for 48 hours to measure moisture content and enable calculation of total flower biomass yield per plant. These flowers were also used to determine pyrethrin content (section 3.2.3). Stems and leaves were also separated and dried in oven at 70^{o}C. Data were analyzed for the significance of main effects and interactions of planting density and family using the Generalized Linear Methods Procedure in SAS v 9.1 (SAS 1990).

3.2.2 Effect of plant density on dry matter partitioning and pyrethrin content in pyrethrum

A second experiment involved a more detailed analysis of DM partitioning at different flower maturity stages (FMS) and pyrethrin content in the same trial described in section 3.2.1 above. The experimental design was simplified to include only planting density as a treatment effect, across random pyrethrum families, which were shown to be not significant (section 3.3.1). Plant processing for DM yield (section 3.2.1) and pyrethrin content (section 3.2.3) was the same as for experiment 1, except that flowers were further sorted into eight FMS stages (Table 1.1). Plant DM

and pyrethrin content data were analysed for the significance of planting density using the Generalised Linear Methods Procedure in SAS v 9.1 (SAS 1990).

3.2.3 Assay for pyrethrin content

Flowers were dried at 50 ˚C for 48 hours, packed into paper bags and stored at -18 ˚C. Extractions were conducted as described by adding 0.5 g powdered tissue with 50 ml hexane (Chromasolv grade), mixing well and sonicating three times for five minutes (one, three and five hours after the extraction started). Samples were then stored for a further 19 hours in the dark. Then, 2 ml of the supernatant was filtered (Alltech 13 mm, 0.45 µm PTFE syringe filter), transferred to a glass vial and stored at -70 ˚C until analysis (Morris *et al.* 2006).

Pyrethrum samples were analysed using the high-performance liquid chromatography (HPLC) using the techniques previously described (McEldowney and Menary 1988; Morris *et al.* 2006; Wang *et al.* 1997). Pyrethrins were chromatographically separated using a 150 mm x 4.6 mm Alltech 'Prevail' silica column with a 0.275% (v/v) isopropanol and 99.725% (v/v) hexanes solvent, at a flow rate of 0.9 ml/min for 8.3 min, which was then ramped straight to 1.1 ml/min until all pyrethrins had eluted. The HPLC system (Waters Alliance 2690) was connected to a Waters 996 diode array detector. Pyrethrum standards were made from a stock solution to concentrations of 0.1 - 2 mg pyrethrin/100 ml to enable the level of extracted pyrethrin in samples to be determined. Pyrethrin content was expressed as percentage total pyrethrins per dry weight of extracted sample.

3.3. Results

3.3.1 Effects of plant density and family on dry matter yield and pyrethrin content

Plant density had significant effects on the DM yield of stems, flowers and total plant biomass and the number of flowering stems per plant and per unit area (Table 3.1). The DM yield of stems, flowers and total plant were greater at high density when expressed on a per unit area basis. Conversely, number of flowering stems and DM yield of stems, flowers and total plant biomass were greater at low density when expressed on a per plant basis (Table 3.1). While significant, the variation in HI per plant ranged between 21 to 25% for low and high densities, respectively (Table 3.1). There were no effects of family or family by density interactions on any of the response variables recorded (Table 3.1). However, there was a trend for families 3 and 50 to produce the greatest stem DW per plant when averaged across densities; yet family 50 had the lowest stem number and DW per unit area (Table 3.1).

3.3.2 Effects of plant density on DM partitioning and pyrethrin content

Plant density had a significant ($P<0.001$) effect on the dry weight of leaves, stems and flowers when expressed on a per plant and per unit area basis (Figures 3.1 A and B). When averaged across densities, approximately 68% of the DM per plant was partitioned to stems, 20% to flowers and 12% to leaves. Although DM per unit area increased with increasing density the DM per plant decreased (Figures 3.1 A and B). The decrease in total plant weight associated with higher densities was largely due to a reduction in stem weight.

In all three density treatments the majority of flowers were at FMS 7 while around 20% or less flowers at FMS 6 or FMS 8 (Figure 3.2 A & B). The number of flowers per plant for all developmental stages was greater at low plant density and decreased with increasing density. However, increased plant number more than compensated

for the lower flower number per plant with more flowers obtained per unit area at high densities (Figure 3.2 A & B).

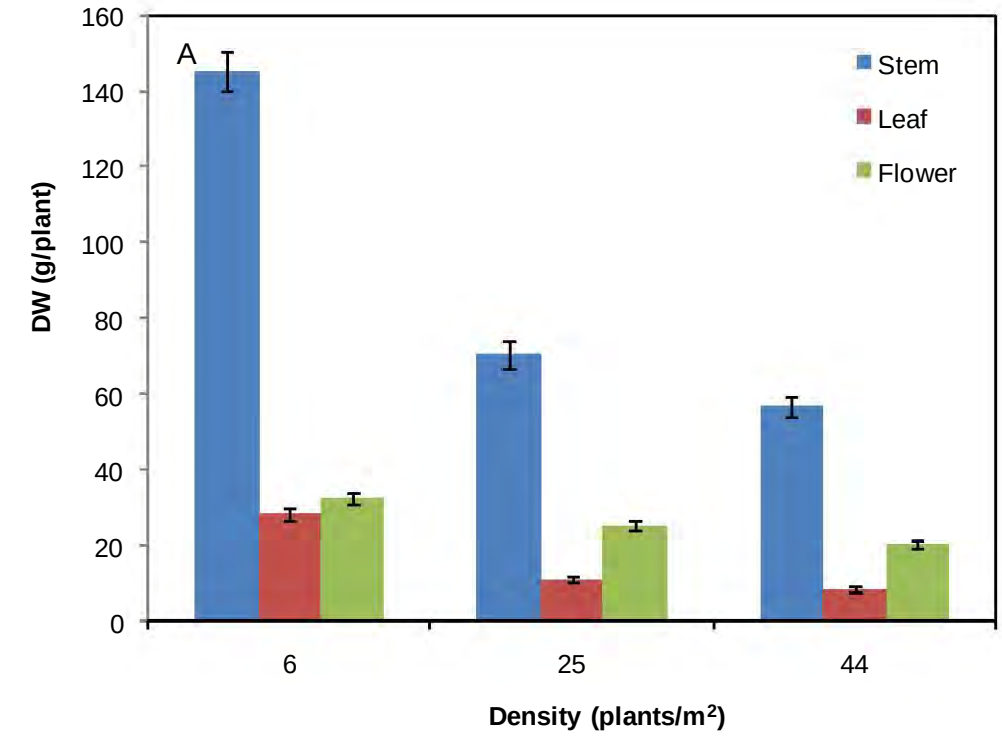
The proportion of flowers at FMS 7 in each of the density treatments was similar on a weight basis (Figure 3.3) and followed the same trend as for flower number (Figure 3.2). However the higher proportion of flowers at FMS 8 at low density suggested that flowers were at a slightly more mature stage of development in this treatment.

Table 3.1 Yield components of 19 pyrethrum plant families per plant and per unit area under low, moderate and high planting densities. The lsd is at *P*=0.05; n.s, not significant.

Density	Stem no.		Stem DM		Flower DM		Total plant DM		Harvest Index
plants/m^2	/plant	/m^2	g/plant	g/m^2	g/plant	g/m^2	g/plant	g/m^2	%
6	79	471	218	1308	73	437	291	1745	25
25	25	624	72	1801	20	499	92	2301	21
44	15	674	47	2051	12	542	59	2596	21
lsd	4	63	18	438	4	58	21	472	1
Family									
1	44	579	134	1681	38	468	171	2153	23
2	39	603	140	2750	36	515	176	3268	21
3	49	768	120	1813	32	518	152	2333	22
4	39	663	110	1823	35	555	144	2383	24
11	41	611	111	1565	33	456	144	2021	22
13	31	516	100	1535	35	516	135	2051	25
15	39	541	137	2160	37	495	174	2658	25
16	41	611	105	1470	38	509	143	1982	26
18	36	546	101	1425	36	492	137	1916	26
19	36	506	115	1419	36	452	151	1868	25
25	40	640	93	1620	30	462	122	2085	25
28	43	623	101	1407	36	479	137	1890	25
32	45	620	128	1839	48	658	176	2500	27
36	40	615	113	1742	32	417	145	2160	23
38	41	601	101	1456	33	482	134	1942	25
39	38	541	125	2420	36	518	161	2938	23
41	39	611	94	1279	32	448	126	1725	26
46	40	595	118	1880	37	485	155	2370	25
50	34	459	88	1390	30	430	118	1821	25
lsd	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Table 3.2 Pyrethrin content on per plant and per unit area basis at low, moderate and high levels of plant densities. The lsd is at P=0.05. n.s, not significant.

Plant density	Pyrethrin 1	Pyrethrin 2	Total	Pyrethrin 1		Pyrethrin 2		Total Pyrethrin	
plants/m^2	%	%	%	mg/plant	g/m^2	mg/plant	g/m^2	mg/plant	mg/m^2
6	0.83	1.05	1.88	267	1.6	368	2.2	636	3.8
25	0.91	1.31	2.22	227	5.6	337	8.4	565	14.1
44	0.87	1.14	2.01	167	7.3	226	9.9	393	17.3
lsd	n.s	n.s	n.s	93	0.2	n.s	0.2	211	4.6



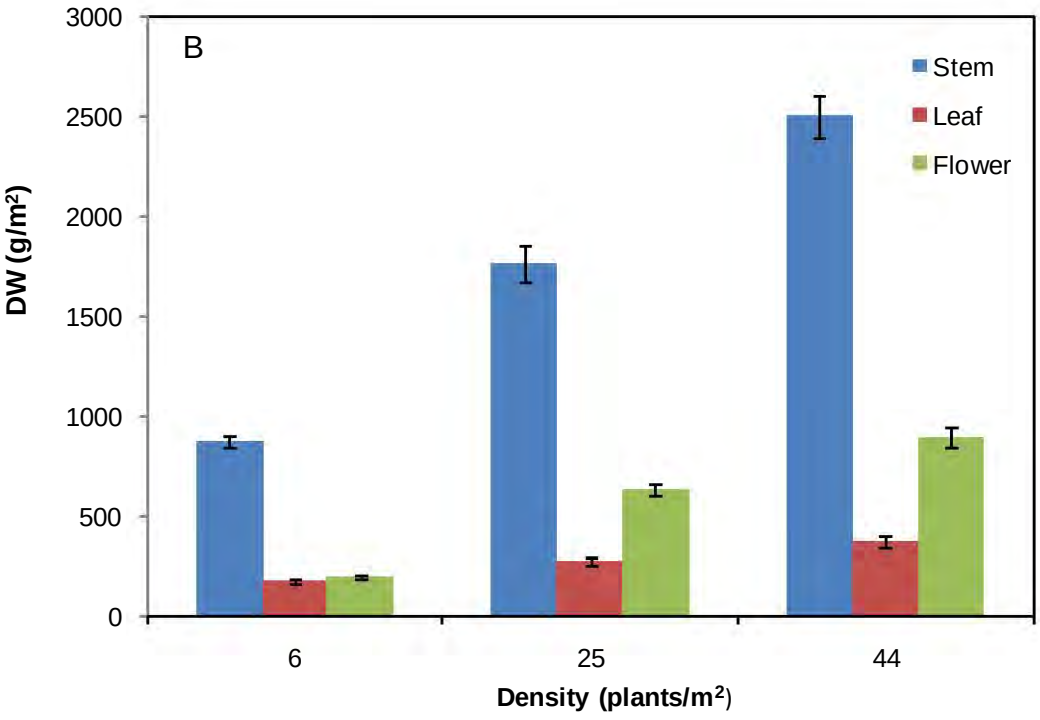


Figure 3.1 Effect of plant density on DM yield of stems, leaves and flowers (A) per plant; and (B) per unit area. Bars represent the SE. Data is from experiment 3.2.2.

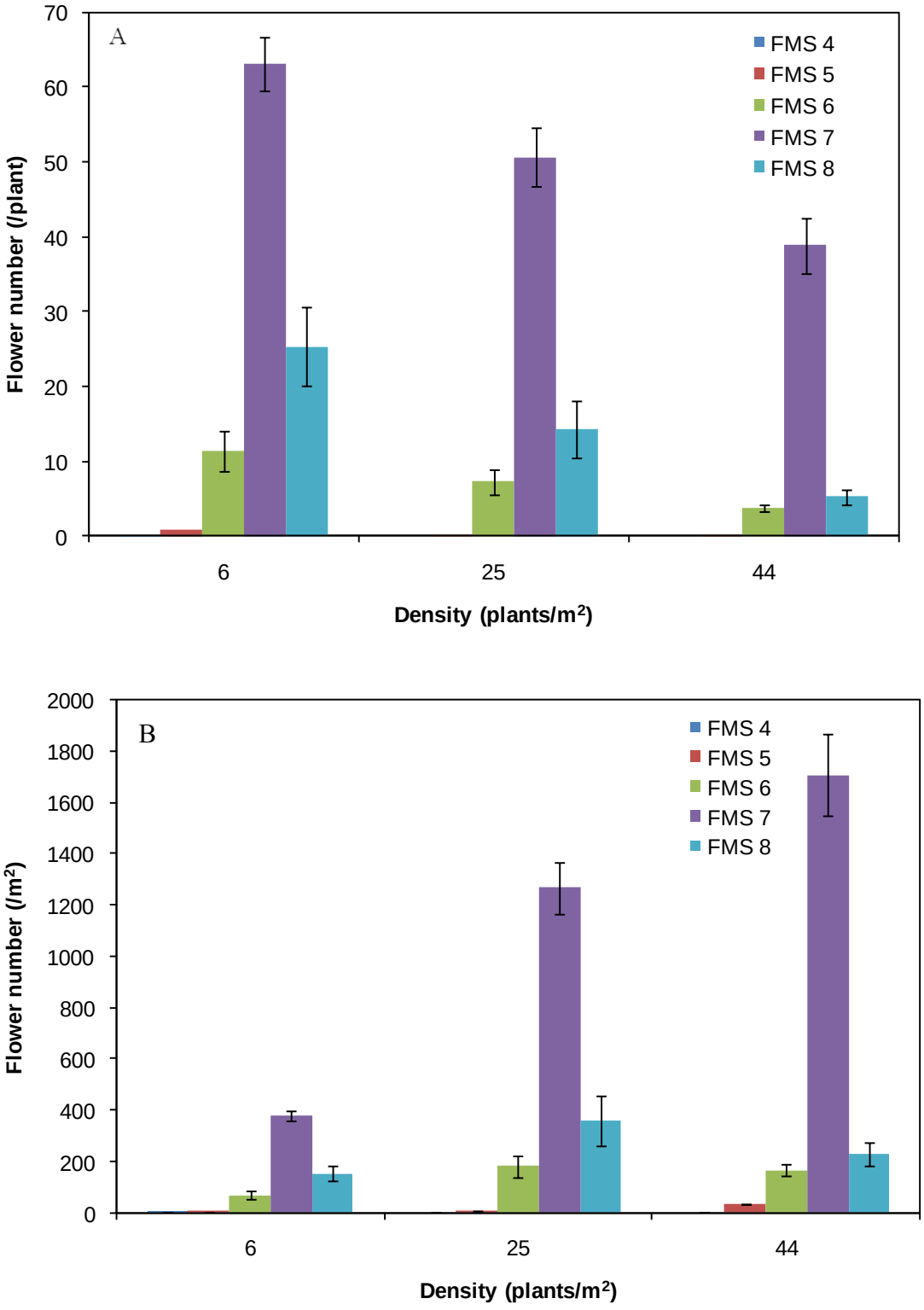


Figure 3.2 The mean number of flowers at each flower maturity stage (FMS) (A) per plant and (B) per unit area, at low, moderate and high levels of plant density. Bars represent the SE.

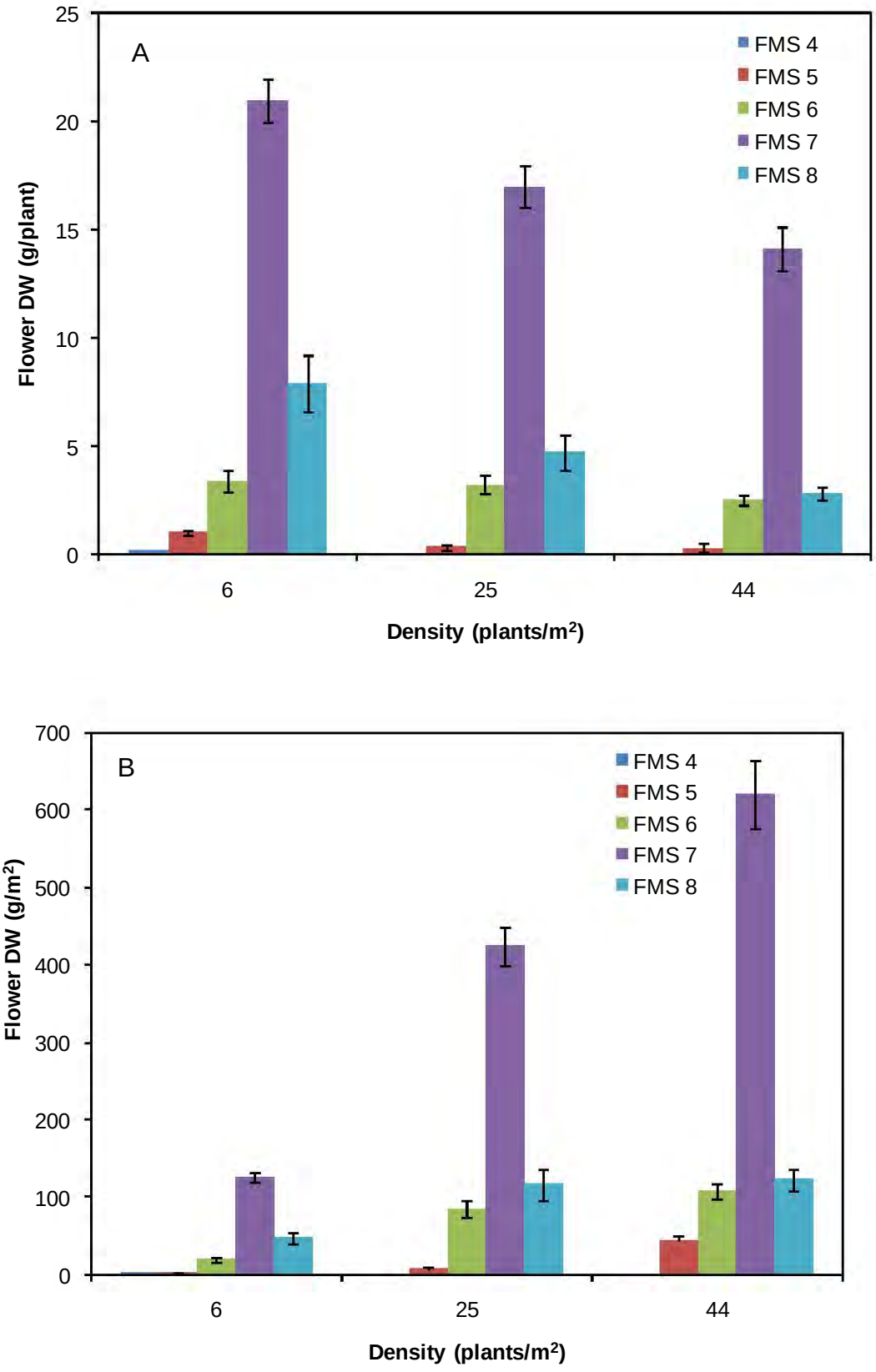


Figure 3.3 The mean dry weight of flowers at each flower maturity stage (FMS) (A) per plant and (B) per unit area, at low, moderate and high levels of plant density. Bars represent the SE.

3.4 Discussion

Results from Chapter 2 suggested that light interception could not fully explain all the variability in flower and total DM yield noted between crops and seasons. It was hypothesised that differences in partitioning of DM to flowers, leaves and stems might be explained by the variability in yield. Potential differences in partitioning between crops might be due to variation in plant stand density. In this chapter, although differences in flower yield were recorded between density treatments, only small differences in DM partitioning to flowers were noted suggesting that any DM partitioning differences between crops are unlikely to be caused by density alone.

Pyrethrum plants grown at low density were larger in size and produced more DM per plant than at high density. Small plant size at high density was compensated by the increased total DM of the crop, consistent with published research (Harper 1977; Papadopoulos and Pararajasingham 1997). When averaged across densities, the majority of DM was partitioned to stems (68%), then flowers (20%) and leaves (12%). Among families there was no statistically significant variation in DM partitioning per plant or per unit area. Pyrethrum breeding programs are in their infancy and therefore the high natural genetic and phenotypic intra-family variation in pyrethrum that could mask any family differences that do exist. The decrease in total DM per plant at high density was thus largely due to a reduction in stem weight and number, consistent with findings reported in 2001 (Fulton *et al.* 2001).

Flower DM had less variation with plant density than stems, suggesting that differences in partitioning associated with plant density were unlikely to explain differences in flower yield noted in Chapter 2 between crops and seasons. It is interesting to note that flower DM yield exceeded previously published reports in the literature. For example, the flower DM yield of 4370 kg/ha at the lowest plant density was 2.5 times that reported for pyrethrum grown at equivalent plant densities in the highlands of Kenya (Wanjala 1991) and is likely to be associated with differences in cultural practices. Flower yields of 5000 kg/ha at the moderate plant density of 16 plants/m^2 slightly (between 4 to 8%) exceeded that previously reported by Fulton *et al*. (2001) in Tasmania. Unlike this study, the field trail reported by Fulton *et al*. (2001) did not use rectangular plant spacing and the lower flower yield may reflect increased inter-row competition between plants for limited resources

(Chung 1990). The data supports the previous recommendation of Fulton *et al*. (2001) for an optimum plant density of between 16 to 39 plants/m^2, which is considerably greater than the 4 – 5 plants/m^2 reported in India (Rao and Singh 1982; Sastry *et al.* 2001).

An additional finding from this study was that FMS was more advanced at low compared with high planting densities, which to our knowledge has not been previously reported. This may have implications for pyrethrin content, which is known to increase with FMI stages but declines at higher FMI due to a growth dilution associated with an increase in flower DM yield (Bhat and Menary 1979). However, pyrethrin content did not vary with plant density, consistent with an earlier report (Fulton *et al.* 2001). At low density individual plants produced more pyrethrins on a plant basis due to the high number of flowers per plant. Conversely, when expressed on a per unit area basis the total quantity of pyrethrins decreased.

In conclusion, differences in DM partitioning between plant components in response to density largely affects stems rather than flower yield or flower pyrethrin content. These results suggest that the differences in flower and total DM yield observed in Chapter 2 between crops and seasons were unlikely to be driven by variation in DM partitioning due to density.

Chapter 4. Effect of watering interval, timing and duration of water stress on yield and pyrethrin content of pyrethrum

4.1 Introduction

Water is a fundamental requirement for plant growth and therefore its availability for uptake during crop growth and development is a major determinant of yield (Boyer 1982). Crops vary in their water requirements for productive growth and yield and have generally been classified as having low, medium or high tolerance to drought stress (Hay and Porter 2006). While annual crops have generally been considered to be more susceptible to water stress than perennial crops, the overall effect of water stress depends on the harvestable component and the timing, degree and duration of stress. Although pyrethrum is regarded as a crop of high water stress tolerance (Chung et al. 1991) there is little known about the effect of water stress during the later stages of development when the harvestable components (pyrethrins) are being synthesised in the flower. Differences in water availability during the flowering phase of crops may explain some variation in flower and pyrethrin yield noted in previous chapters.

Plant soil water availability has been shown to be influenced by soil physical and chemical properties and can be measured at a point in the soil profile directly using tools such as soil moisture probes or what the plant is experiencing by taking pre-dawn leaf water potential measurements (Parker 2010). Water moves according to water potential gradients and therefore during a period of drought stress the available moisture for plant uptake in soil is reduced; this in turn reduces the plant water potential and cell turgor. The plant responds to short term changes in water availability by reducing normal physiological functions and growth (Tezara *et al.* 1999). Periods of water stress have been shown to reduce meristematic tissue growth, photosynthesis and therefore biomass accumulation (Connor and Jones 1985; Hay and Porter 2006; Singal *et al.* 1984), partitioning and harvest index (Hay and Porter 2006).

Several studies have been conducted in field conditions in Tasmania on the impact of drought stress on pyrethrum production. An observational study conducted over three production seasons (1992/3, 1993/4, 1994/5), and involving in excess of fifty commercial pyrethrum crops grown in Tasmania, claimed that water stress occurring September/October (early flower bud development) results in a lower number of flowers at harvest, while drought stress occurring during November/December (late in flower development) reduced the total flower weight and possibly pyrethrin content (Rand, 1990). However, there was no evidence of statistical analysis of the data set in the report and hence the claims made appear to be qualitative rather than quantitative. These observations were consistent with previous comments made by Brown and Menary (1994b) that pyrethrum plants grown under elevated temperature and inadequate moisture during flowering developed as a faster rate and hypothesised that this could lead to reduced pyrethrin synthesis and yield.

In the field study by Rand (1990) soil water availability was monitored in each of the commercial pyrethrum crops at a range of depths in the soil profile. This enabled the author to demonstrate the deep-rooted nature of pyrethrum and where soil water was extracted from within the soil profile of different soil types throughout the growth and development of the crop. In the study by Rand (1990) it was noted that pyrethrum plants rarely showed visual moisture stress symptoms, even when what would typically be deemed high moisture stress conditions, and that the crop could extract water from as deep as 120 cm in the soil profile in deep friable soils. The ability of the crop to survive high water deficits and still give moderate yields is consistent with a preliminary study (Chung *et al.* 1991).

There is a lack of quantitative information on the impact of water stress during key stages of flower development in pyrethrum on flower biomass and pyrethrin synthesis and accumulation. Although there is some evidence to support the hypothesis that water stress reduces pyrethrum flower DM and pyrethrin yield, little is known of drought severity sensitivity of the plant. The aim of this research chapter was to determine the effect of water stress at key stages of flower development and the degree and duration of water stress on the growth, yield and pyrethrin content of pyrethrum.

4.2 Materials and methods

4.2.1 Cultural details

The experiment was conducted at the Horticulture Research Centre, University of Tasmania, Hobart. The timing of the experiment was in parallel with the commercial pyrethrum production in Tasmania from August to December 2009. Pyrethrum seedlings, cultivar Pyper, were grown in 4.5 L pots containing a standard potting mix of sand (50:50 (v/v)), peat moss (40 g) and ground limestone (40 g). All the seedlings were grown under ambient temperature in a shade house up to FMS 1 (flowering stage) and then transferred to a glasshouse. Plants used in the experiment were selected on the basis of uniformity in plant size. Figure 4.1 shows the temperature and humidity of the glasshouse under which the trial was conducted.

4.2.2 Trial design

To assess the effect of water stress at key stages of development, two experiments were conducted involving watering interval to impose different levels of stress, the duration of water stress and timing of stress. The first experiment was a 3 x 3 factorial design consisting of treatments three levels of watering interval (3, 4 and 5 days) for a 10 day period at three flower maturity stages (early flowering stage, FMS 2; mid flowering stage, FMS 4; late flowering stage, FMS 6) in a randomised block design with 11 replicates (pots). The second experiment was conducted concurrently. The experiment consisted of a 2 x 2 factorial design of treatments with two levels of watering interval (3 and 5 days) for a 20 day period at two flower maturity stages (early flowering stage, FMS 2 and mid flowering stage, FMS 4) in a randomised block design with 11 replicates (pots). Eleven control pots that were watered daily were included in both experiments. The FMS stage for individual plants was based on the primary flowering stem and treatments were applied when at least five replicate plants reached the target FMS.

At the commencement of treatment, soil was brought to field capacity by soaking the pots in a container of water for one minute and allowing them to drain. The weight of pots at field capacity was recorded. Pots were then weighed at the end of the watering interval (1, 3, 4 or 5 days) and returned to field capacity.

Direct measurements included water use. Soil water potential was determined at the end of each watering interval by deriving an understanding of the relationship between pre-dawn leaf water potential and soil moisture content. In addition, plant stress response at different soil moisture contents was extrapolated from the relationship between chlorophyll fluorescence and soil moisture content. All plants were harvested on the same day when the flowers reached a stage consistent with commercial practices and plant dry matter yield and pyrethrin content (for experiment 1) determined.

4.2.3 Plant growth and dry matter yield

Plant growth and development measurement at harvest included the number of primary, secondary and tertiary flowering stem, the total number of flowers for each of the eight FMS stages and the plants were divided into leaves, stems and flowers. Leaves and stems were dried at 70 ˚C and flowers at 50 ˚C for 48 hours and weighed. The analysis of total pyrethrin content and ratio of pyrethrin 1 to pyrethrin 2 was done using High Performance Liquid Chromatography (HPLC) using the procedure described in section 3.2.3.

4.2.4 Measurement of soil water potential

Estimated soil water potential (ESWP) for treatments was based on an understanding of the relationship between pre-dawn leaf water potential (PLWP) and % pot water loss to avoid destructive measurements on experimental pots. Twelve randomly selected plants representative of the experimental population were selected at FMS 2, watered to field capacity as described above, placed in a dark room overnight. Soil water potential (SWP) was measured the following morning between 6:30 to 8:30 am on one leaf petiole per pot and plants were returned to standard growing conditions. This was repeated for 10 consecutive days. The SWP of pyrethrum plants was

measured using a Pressure Chamber (Model 615, PMS Instruments Co.) and the weight of the pots at the same time to determine the relationship between SWP and % pot water loss.

4.2.5 Chlorophyll fluorescence

Chlorophyll fluorescence for treatments was based on an understanding of the relationship between chlorophyll fluorescence and % pot water loss, using the same 12 plants from 4.2.4. The chlorophyll fluorescence was measured using a FluoroPen (Qubit Systems, Canada), which measured the quantum yield (QY) of photosystem II efficiency of fully-expanded leaves, which was equivalent to Fv/Fm (Fracheboud 2007). The average of five readings was recorded for each plant.

4.2.7 Data analysis

Data were analysed for the significance of main effects and interactions of watering interval and FMS stage for pot water use, ESWP, estimated chlorophyll fluorescence, yield and (for experiment 1) pyrethrin content using the general linear model (GLM) procedure in SAS v9.1 (SAS 1990). Non-linear regressions were used to describe the relationship between estimated soil water potential ESWP and estimated chlorophyll fluorescence with % pot water loss.

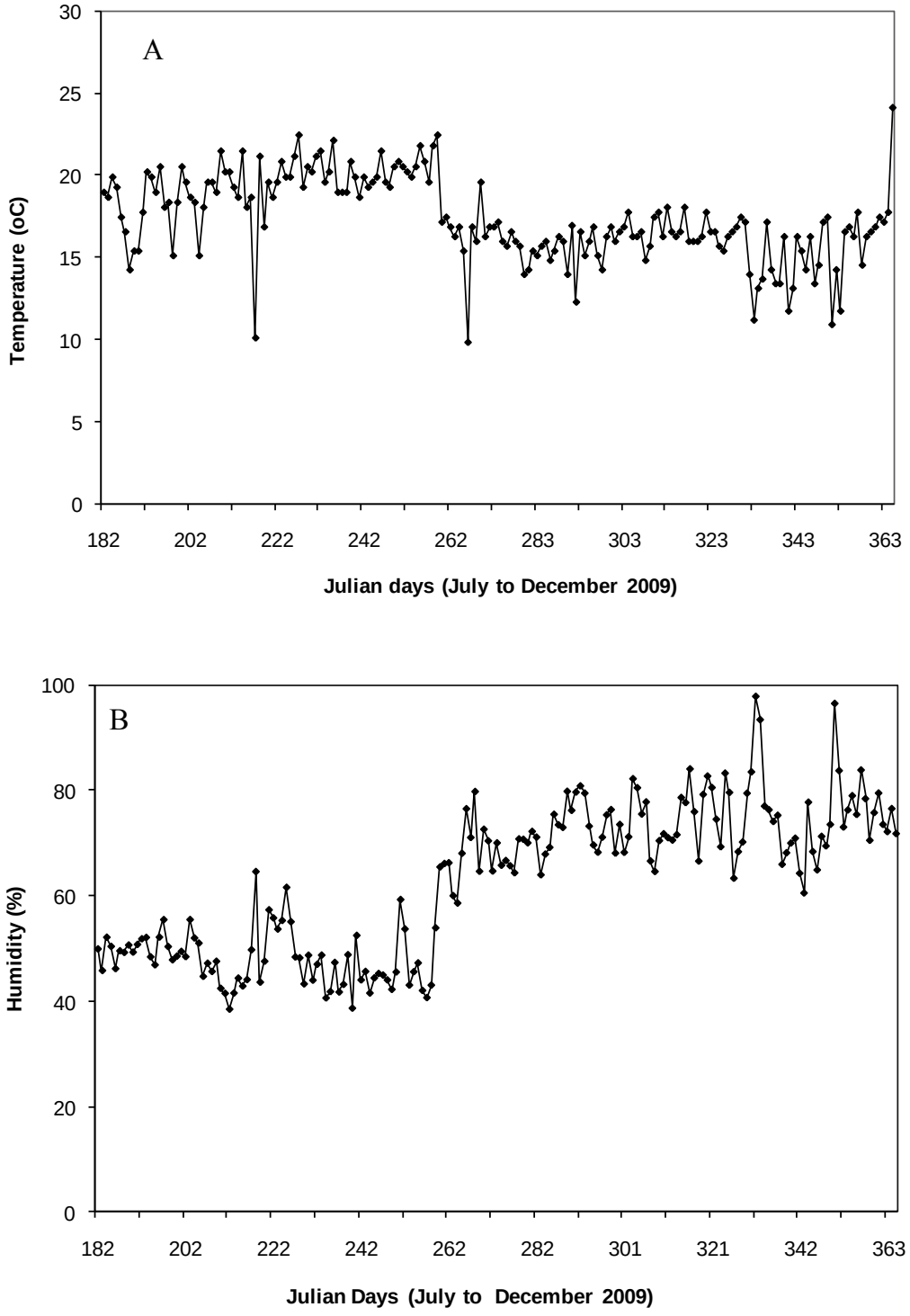


Figure 4.1 Average daily (A) temperature and (B) humidity of the glasshouse from July to December 2009.

4.3 Results

4.3.1 Soil water potential

Soil water potential (SWP) decreased exponentially with cumulative % pot water loss (Figure 4.2). The average SWP at field capacity was -0.13 MPa.

Representative examples of drought stress treatments applied at FMS 4 on ESWP are presented in Figure 4.3. For plants watered daily the ESWP typically declined to -0.3 MPa. For pots watered every third day, ESWP declined to -1.00 to -1.45 MPa (soil moisture loss of 20 to 35%). Pots watered every fifth day the ESWP were as low as -1.26 to -1.72 MPa (soil moisture loss of 30 to 40%).

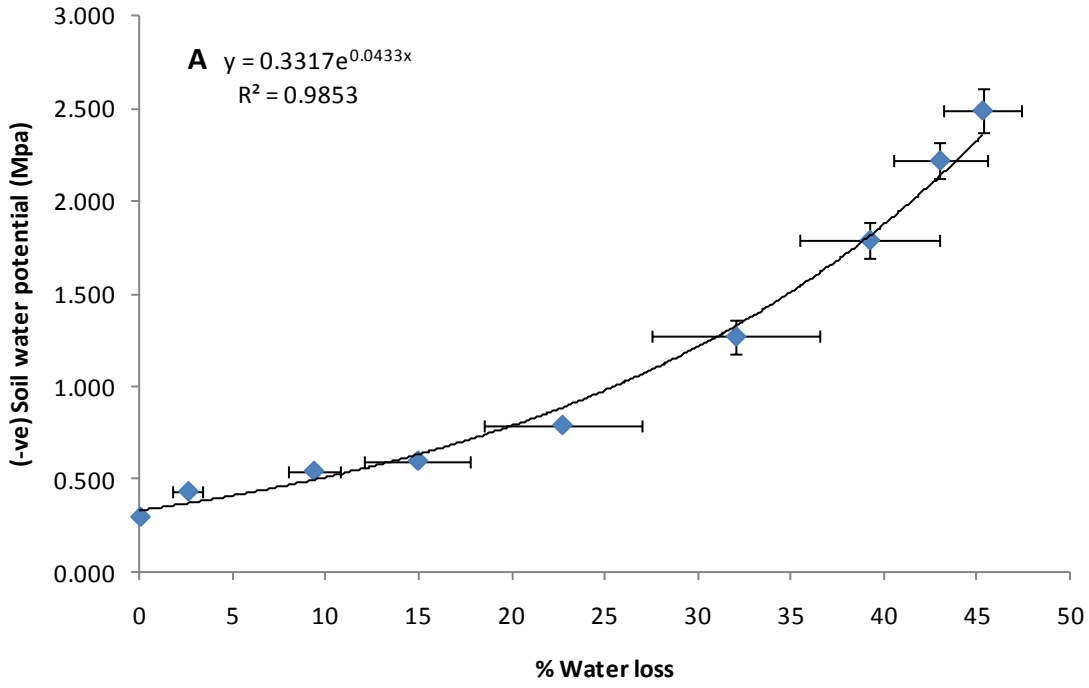


Figure 4.2 Relationship between changes in % soil water loss with soil water potential of pyrethrum plants grown in pots. Bars represent the SE.

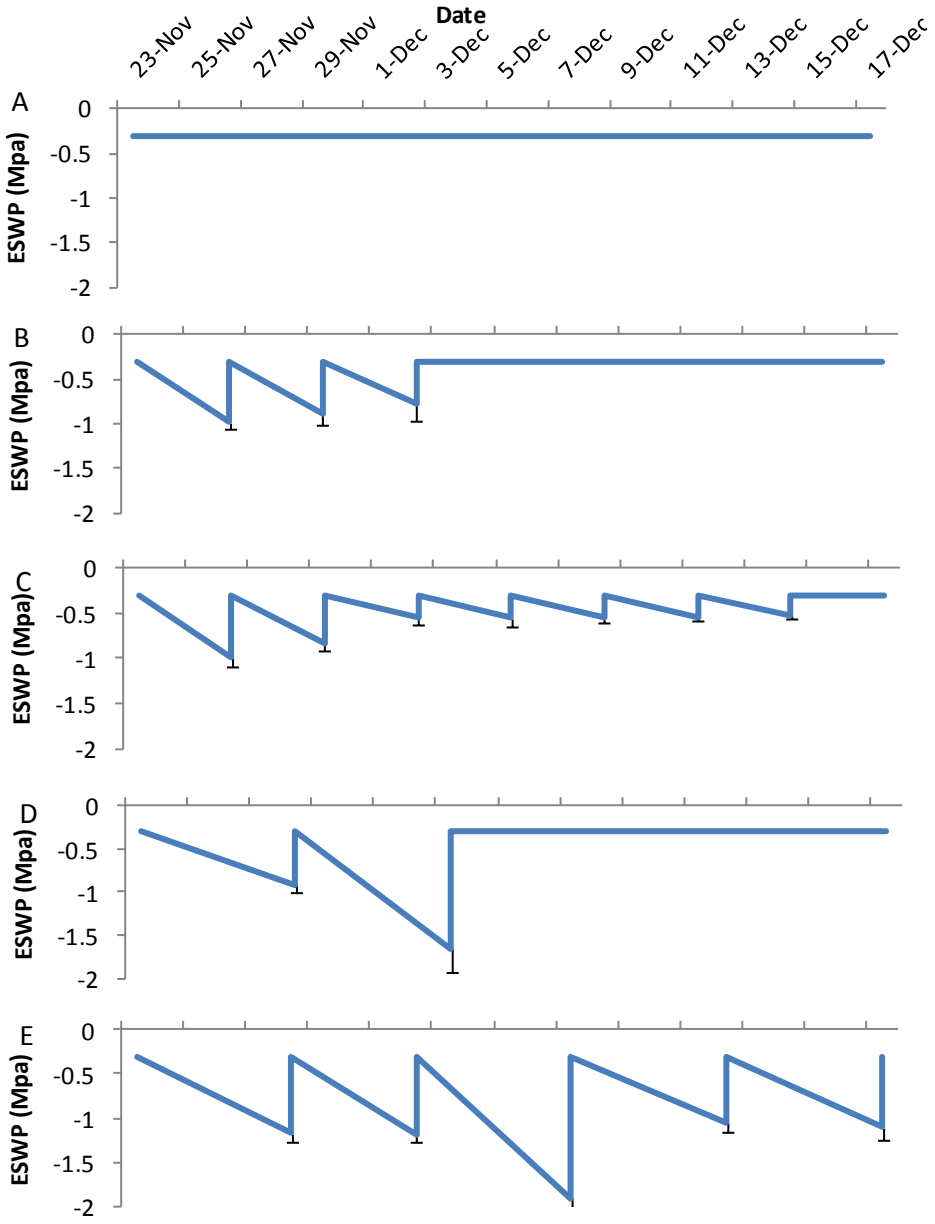


Figure 4.3 Change in ESWP over time in response to selected drought stress treatments applied at FMS 4: A) control; B) 3 d watering interval for 10 d; C) 3 d watering interval for 20 d; D) 5 d watering interval for 10 d; and E) 5 d watering interval for 20 d. Bars represent the SE.

4.3.2 Chlorophyll fluorescence measurement

There was a negative polynomic relationship between cumulative % pot water loss and chlorophyll fluorescence (Figure 4.4). The average chlorophyll fluorescence was 0.785 nm at field capacity and this decreased to 0.670 at 45% water loss.

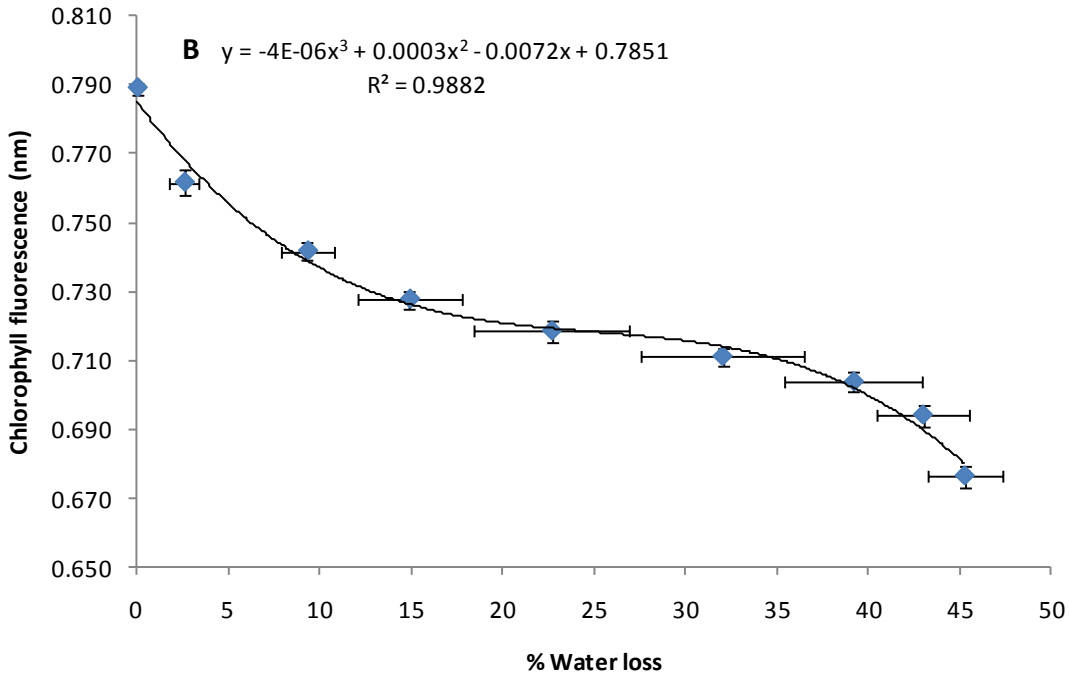


Figure 4.4 The relationship between % soil water loss and chlorophyll fluorescence of pyrethrum plants grown in pots. Bars represent the SE.

4.3.3 Effect of FMS stage, severity and duration of water stress on crop biomass, yield and pyrethrin content

For experiment 1, the watering interval and FMS stage of commencing water stress treatments over a period of 10 days had significant effects on ESWP, estimated chlorophyll fluorescence, flower numbers at FMS 6, 7 and 8, leaf and total DM but had no effect on the pyrethrin content (Table 4.1 and 4.2). Applying the water stress treatments at FMS 2, regardless of watering interval, had the highest DM yield of leaves, stems and flowers and flower numbers compared with FMS 4 and FMS 6

(Table 4.1). Reduced total plant dry weight in response to the drought stress treatments was mainly caused by decreased leaf dry weight (Table 4.1).

For experiment 2, the watering interval and FMS stage of commencing water stress treatments over a period of 20 days had significant effects on ESWP, estimated chlorophyll fluorescence, number of flowers at FMS 8 and flower DM (Tables 4.1 and 4.3). ESWP was more negative and estimated chlorophyll fluorescence reduced when the watering interval was increased from 3 to 5 days (Table 4.1). Water stress that was applied during mid-flower development at FMS 4 resulted in fewer flowers reaching FMS 8 by harvest and a smaller flower DM (Table 4.1).

4.3.4 Effect of FMS stage on DM yield and number of flowers

The effects of applying water stress for 10 d at different FMS stages on the flower number at FMS 6, 7 and 8 and DM yield at harvest are presented in Figure 4.4A and B. The majority of flowers (around 60%) had reached FMS 8 in all FMS treatments and the control by harvest. However, close to 50% of flowers delayed progress when water stress was applied at FMS 6 and were immature at harvest (Figure 4.4A).

For DM partitioning, water stress reduced flower DM by around half and increased stem DM by 25% compared with the control. Among FMS treatments, plants produced more leaf and total DM when water stress was applied at FMS 2 than at later stages of development (Figure 4.4B). There was no significant difference in flower or stem DM with FMS treatment.

4.3.5 Effect of watering interval and FMS stage on estimated soil water potential and chlorophyll fluorescence and pot water content of pyrethrum

The effects of applying water stress over a 10 day period using different watering intervals and applied at different FMS stages on pot water content, ESWP and chlorophyll fluorescence are shown in Figure 4.5A, B and C. ESWP was on average -1.0 MPa (25% pot water content) and -1.5 MPa (30% pot water content) at the end of the 3 and 5 day watering intervals, respectively, across all FMS stages (Figure

4.5A and B). In contrast, ESWP and pot water content increased from early to late FMS stages for the 4 d watering interval. Estimated chlorophyll fluorescence followed a similar trend to ESWP (Figure 4.4C).

Table 4.1 Effect of drought treatments of watering interval (daily or every 3, 4 or 5 d), duration of stress (20 or 30 d) and development stage when stress was applied (FMS2, 4 or 6) on estimated soil water potential (ESWP) and chlorophyll fluorescence (Fv/Fm), components of yield and pyrethrin content. The lsd, for a one-way ANOVA, is at P = 0.05; n.s, not significant. Data are for both experiments 1 and 2. The experimental design did not allow for statistical analysis of the full three-way interaction.

Treatments			Pot	ESWP	Fv/Fm	Number of stems		Number of flowers				Dry matter (g/plant)				Pyrethrin (%)	
Watering interval (d)	Duration (d)	FMS stage	water content (%)	(MPa)	(nm)	Start	Harvest	FMS 6	FMS 7	FMS 8	Total	Flower	Leaf	Stem	Total	1	2
1	0	n/a	0.00	-0.34	0.780	70	67	3	26	38	77	17.3	24.0	33.0	74.4	1.15	1.18
3	10	2	24.5	-1.03	0.729	61	54	7	14	33	60	14.2	22.9	32.0	69.2	1.14	1.39
3	10	4	27.6	-1.45	0.729	56	57	15	11	31	68	15.8	18.0	33.5	67.3	1.45	1.19
3	10	6	25.2	-1.01	0.729	62	55	29	7	20	64	17.9	17.0	32.6	67.6	1.19	1.46
3	20	2	23.9	-1.00	0.731	64	57	10	16	32	67	15.4	20.8	31.2	67.5	-	-
3	20	4	23.6	-0.95	0.730	68	49	12	11	25	61	13.5	19.9	31.8	65.2	-	-
4	10	2	23.4	-1.02	0.730	56	55	9	14	33	61	15.9	22.0	35.0	72.9	-	-
4	10	4	33.5	-1.83	0.689	53	46	10	8	26	53	13.9	17.0	28.9	59.9	-	-
4	10	6	38.9	-1.43	0.724	63	53	20	9	19	62	15.8	16.7	30.7	63.3	-	-
5	10	2	30.9	-1.71	0.716	53	52	1	10	40	58	13.5	21.4	32.0	67.0	1.15	1.34
5	10	4	27.9	-1.72	0.708	56	50	7	14	29	55	14.3	20.3	29.8	64.4	1.12	1.42
5	10	6	32.2	-1.42	0.727	56	47	23	9	18	58	13.6	16.4	27.0	57.1	1.29	1.64
5	20	2	28.4	-1.26	0.728	67	59	6	8	45	64	16.8	22.6	37.6	77.0	-	-
5	20	4	33.7	-1.52	0.722	53	46	17	9	19	52	13.4	19.0	29.9	62.3	-	-
lsd			4.0	0.28	0.015	n.s	n.s	8	8	12	n.s	n.s	3.2	n.s	9.9	n.s	n.s

Table 4.2 Mean squares of treatment main effects and interactions for watering interval (W; every 3, 4 or 5d), and development stage when stress was applied (FMS; 2, 4 or 6) for 10 d duration for estimated soil water potential (ESWP), estimated chlorophyll fluorescence (Fv/Fm), components of yield and pyrethrin content. Data are for experiment 1.

Treatment	d.f	Pot water (%)	ESWP (MPa)	Fv/Fm	Number of stems		Number of flowers			Dry matter (g/plant)					Pyrethrin (%)	
					Start	Harvest	FMS 6	FMS 7	FMS 8	Total	Flower	Leaf	Stem	Total	d.f	1	2
block	10	34	0.16	0.00040	294	473	159	108	283	309	22.2	21.3	136.5	318.2	10	0.090	0.114
W	2	335***	2.68***	0.00202*	170	460	350*	2	77	267	40.6	6.9	80.6	224.1	2	0.005	0.089
FMS	2	283***	0.96**	0.00327**	247	59	2857***	141	2258***	94	15.7	253.4***	76.1	471.2**	2	0.045	0.380
W x FMS	4	241***	0.61**	0.00154*	85	207	85	65	86	138	18.8	15.5	53.2	167.3	2	0.019	0.083
Error	80	26	0.15	0.00047	263	277	105	96	255	261	15.0	13.7	42.6	118.5	37	0.098	0.197

*,**,*** indicates statistical significance at P = 0.05, 0.01 and 0.001, respectively

Table 4.3 Mean squares of treatment main effects and interactions for watering interval (W; every 3 or 5 d), and development stage when stress was applied (FMS; 2 or 4) for 20 d duration for estimated soil water potential (ESWP), estimated chlorophyll fluorescence (Fv/Fm) and components of yield. Data are for experiment 2.

Treatments	d.f	Pot water (%)	ESWP (MPa)	Fv/Fm	Number of stems		Number of flowers				Dry matter (g/plant)			
					Start	Harvest	FMS 6	FMS 7	FMS 8	Total	Flower	Leaf	Stem	Total
block	10	14	0.03	0.00002	265	150	111	120	136	129	7.3	13.6	24.9	79.7
W	1	578***	1.87***	0.00031**	402	426	2	222	124	1	4.4	1.8	53.6	116.4
FMS	1	68*	0.12	0.00013*	231	882	471	31	2978**	1364	79.2*	56.9	139.5	798.4
W x FMS	1	82*	0.25*	0.00005	864	102	218	102	1080*	84	5.7	19.2	190.0	423.5
Error	30	12	0.03	0.00002	519	453	115	131	173	349	18.8	15.5	94.4	213.6

*,**,*** indicates statistical significance at P = 0.05, 0.01 and 0.001, respectively

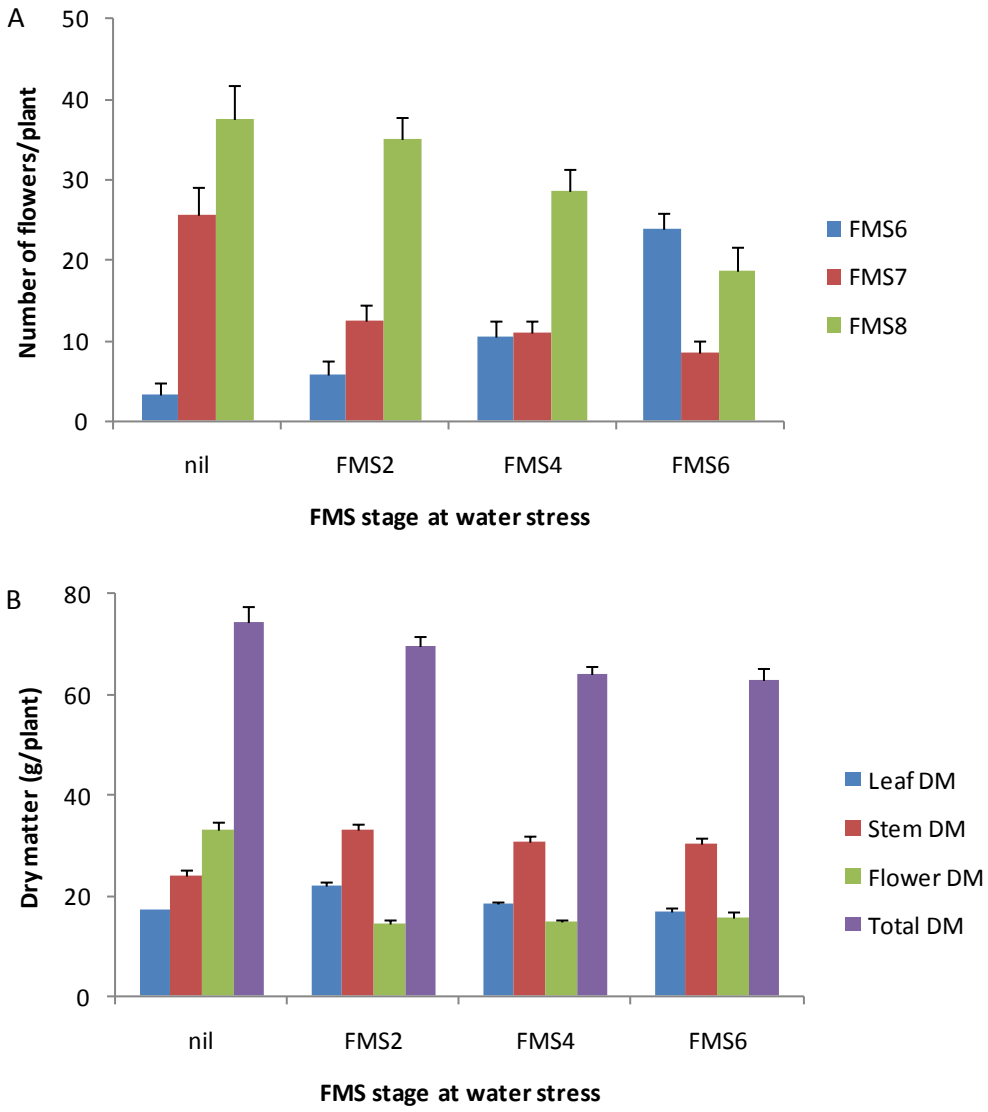


Figure 4.4 Effect of stage of development (control versus FMS 2, 4 or 6) when water stress was applied on A) number of flowers/plant at FMS 6, 7 and 8 at harvest; and B) leaf, stem, flower and total DM per plant. Bars represent the SE, n = 11. Data are from experiment 1.

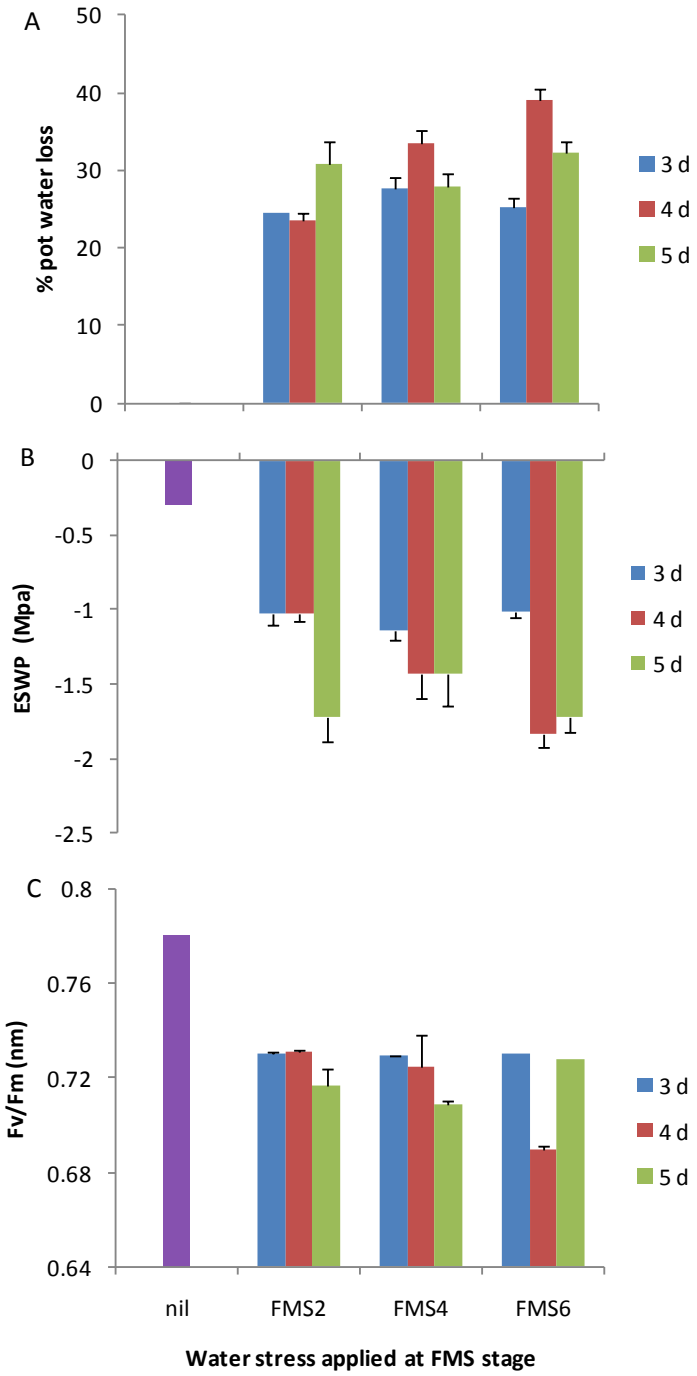


Figure 4.5 Interaction between effects of stage of development when water stress was applied (nil versus FMS 2, 4 or 6) and watering interval (3, 4 or 5 d) for A) estimated soil water potential; and B) estimated chlorophyll fluorescence (Fv/Fm). Bars represent the SE, n = 11. Data are from experiment 1.

4.4 Discussion

Little variation in flower yield and pyrethrum between crops was explained by light interception in Chapter 2 and plant density in Chapter 3. The present chapter explored the effect of water stress during flowering, the key phase of pyrethrin synthesis (Brown 2005), on flower yield and pyrethrin content. The experiments explored a hypothesis that water stress during flowering limits flower DM accumulation and synthesis of the secondary metabolites.

Pyrethrum, with its perennial habit and deep root system (Brown 2005), is regarded to be tolerant of drought stress. At present, standard agronomic practice is to irrigate the crop at key stages of development such as establishment and flowering. Although there have been a number of observational studies (Chung *et al.* 1991; Rand 1990), no detailed studies of how the plant responds to water stress during the flowering period have previously been documented. The present study has shown that plants continued to partition resources to the flowers and accumulate pyrethrins even under short periods of water stress as low as a soil water potential of -1.8 MPa. The relationships between chlorophyll fluorescence readings and water loss confirmed that the watering intervals most likely would have mildly stressed the plants and reduced photosynthesis.

The plants respond to water deficits by reducing dry matter partitioning to leaves and stems. Water stress did not significantly affect pyrethrin accumulation even when it corresponded to the main phase of pyrethrin synthesis between FMS stage 1 and 4 (Groom 2003). While there appears to be a lower total pyrethrin yield when the drought treatments were applied at FMS 2, a significant result may have required further replication. Therefore drought stress during the early stages of flowering could significantly inhibit pyrethrin synthesis. One of the challenges of working with a relatively new crop is the high level of phenotypic variability (Groom 2003). This variability was minimised by selecting plants of relatively uniform size and development prior to commencement of the experiment.

In these factorial experiments drought applied at different FMS stages had a greater effect on the plant growth and development parameters measured compared with the other treatments (watering interval and duration of water stress). In general, drought

applied at FMS 2 did not reduce plant biomass accumulation to the same extent as drought applied at FMS 4 and 6. However, drought applied at this stage of development had a slight influence on pyrethrin synthesis. The higher biomass accumulation of plants that were drought stressed at FMS 2 compared with FMS 4 and 6, maybe the result of these plants having a longer recovery period. There was also a trend towards drought applied at later flowering stages to delay flower maturation, as shown by the increased proportion of flowers at FMS 8 at harvest in plants that received drought stress at FMS 6. This latter finding is contrary to field observations where drought stress hastens flower development (Brown 2005). The differences between the findings in this report and the findings of Brown (2005) may be associated with the degree of the stresses imposed with potted plants experiencing short and severe drought stress compared with the field. The ability of the plants to recover following drought stress is consistent with past irrigation trials in field conditions that showed pyrethrum plants recovered quickly from stress when watering was resumed (Chung *et al.* 1991).

In conclusion, the impact of drought stress during flowering on pyrethrum yield varied with stage of plant development. Drought applied at the early stages of flower development had minimal impact on yield, but may have reduced pyrethrin accumulation. In contrast, drought applied at later stages of plant development resulted in decreased total plant DM yield, with little effect on flower DM or pyrethrin content.

Chapter 5. General Discussion

The lack of predictability and consistency in pyrethrin yield within and between growing seasons in pyrethrum crop is a major problem for the Tasmanian pyrethrum Industry. The pyrethrin yield of the pyrethrum crop is composed of two main yield components; flower DM yield and the pyrethrin concentration in the flowers. The total flower DM weight is made up of the number of flowers and weight of individual flowers, with the number of plants and flowering stems per unit area influencing these yield components (Parlevliet 1970). The total pyrethrin yield is made up of pyrethrin concentration or assay in the flowers and the DM yield weight of flowers. The lack of consistency in pyrethrin yield may be due to variability in the main yield components of total DM and pyrethrin yield concentration, both of which may be influenced by plant and environmental factors as well as genetic and management factors within crops and seasons.

Crop management practices, genetic and environmental factors have been shown to influence the two main pyrethrin yield components. For example, in this study plant density affected DM yield but pyrethrin concentration was shown to be stable across the range of densities tested. In previous studies (Fulton *et al.* 2001; Sastry *et al.* 2001) DM yield has been shown to be affected by irrigation management, but the capacity of the crop to achieve harvestable yields at water deficits up to 90 mm has led to the conclusion it is more water stress tolerant than other crops such as poppies (Brown 2005; Chung 1987; Chung *et al.* 1991). This conclusion was supported by the results from the pot trial undertaken in this project, with small changes in yield found across a broad range of water stress timing, duration and severity treatments. The small changes in yield that have often been found in trials examining single factor variables suggest a plasticity in formation of yield components, for example the increase in stem number per plant that occurs at lower plant density. The large differences in yields found between crops and between seasons may therefore be linked to compounding effects of multiple factors rather than a single dominant factor.

The pyrethrum florescence contains the majority of the plant's active compound, approximately 1-2% pyrethrin (Casida and Quisad 1995). Over 90% of the total pyrethrin content was observed to be derived from achenes or disc florets (Chandler 1951) and the accumulation of the pyrethrin in the achenes has been closely related with the development of oil glands in the flowers (Bhat and Menary 1979). The pyrethrin content per flower is therefore determined by the FMI and genetic differences of clones. The flower pyrethrin concentration increased with FMI stages but declines at higher FMI as accumulation of DM yield increases to high FMI (Bhat and Menary 1984b). Pyrethrin content per flower therefore increases rapidly during early FMI stages before a plateau at later stages, while flower dry weight continues to increase at these later stages. Differences in the relative rates and timing of pyrethrin and dry matter accumulation under different treatments, combined with the variability in stem and flower number associated with the yield plasticity in pyrethrum, makes prediction of yield based on individual management factor or environmental factors very difficult.

The differences in flower DM yield were well illustrated in the data presented for commercial pyrethrum crops in the 2006, 2007 and 2008 seasons. While differences in the canopy coverage of the crops and radiation intercepted partially explained the variability in the crops yield, other factors were clearly impacting on yield. At a gross level when separating good and poor commercial crops, the low yielding crops were those with low canopy coverage, resulting in lower interception of light during the growing season and consequently low DM yield. Variation in soil moisture level, pyrethrin content of seed used in sowing and effects of fungal diseases and frost events occurring in the field may all have contributed to the differences in canopy coverage.

Great variation in the total radiation received within crops between the 2006, 2007 and 2008 seasons was noted. The variation is due to cloud cover, and differences in other environmental factors such as rainfall, humidity and temperature also occurred between seasons. The differences in rainfall and temperature may have also contributed to differences in yield between seasons through, for example, changes in the rate of pyrethrin accumulation or duration of pyrethrin accumulation or the FMI stage at which the pyrethrin development occurred and ceased. The lack of correlation between

radiation interception and yield between seasons suggests that these or other factors have a significant impact on yield. In addition, it was assumed that variation in leaf area index between crops and seasons would not be sufficient to mask any relationship between radiation interception, calculated on the basis of ground cover, and DM yield. Given the weak relationship found in this study, this assumption should be tested by assessment of radiation interception using leaf area index measurements. Also, the timing of measurements for each crop was consistent within and between seasons, but the timing of flower development was noted to vary between sites and seasons. Thus, comparisons across a common time period did not correspond to a common crop developmental phase. The measurement of radiation interception by the canopy on an area or volume basis was done over the entire growing season but it is possible that only light received in the period of rapid growth and flower development, FMI 200-600, was critical to flower DM yield. It is suggested that future research look at light interception between key plant stages rather than over a calendar date range to establish if a relationship between radiation interception and yield exists within crop developmental phases.

Plant density was found to have the largest effect on the total crop DM yield. The total DM was high in high plant density crops while low in low density. Planting densities that did not lead to full ground cover during the growing season had significantly lower radiation interception and DM yield. At high density, the dense canopy coverage led to maximum radiation interception and low light levels within the canopy. The plants respond to the low level of light by reducing flowering stem number and stem branching. This reduced number of flowering stems and flowers per stem and per plant, but because of high number of plants on per unit area the numbers of flowering stem and flowers were high. High stem density increased the canopy coverage as branching into secondary and tertiary stems with leaf growth increased vertical and horizontal interception of light and therefore flower DM yield. The capacity of pyrethrum to vary stem density provides plasticity in response to variations in plant density and therefore for similar yields to be obtained across a broad plant density range.

At low density, the total DM was high on a per plant basis but low on a per unit area basis. The low number of plants per unit area and less dense canopy permitted high total radiation interception per plant, increased plant size and encouraging more

vegetative growth with a high number of flowering stem number, flower number and flower DM yield on a per plant basis but low on a per unit area basis. In addition the low number of plants per unit area allowed weeds to grow and had high competition with the plants for nutrients, water and light which is likely to have resulted in a further reduction of crop DM yield.

Changes in flower DM content and pyrethrin content were noted with changes in flower maturity. The results were consistent with those of (Fulton *et al.* 2001). In previous density trials, pyrethrin concentration remained constant at around 2.30% in all levels of densities, with pyrethrin yield per flower being related to flower DM yield and maturity stages (Rao and Singh 1982).The flower DM was high at the later stages of FMI while the pyrethrin yield increased during early flower development and reached a peak at mid FMI stages corresponding to individual flower stages of FMS 4 to FMS 5. At later FMI stages, FMS 7 and FMS 8, the pyrethrin concentration in flowers declines as DM accumulation proceeds. The differences in patterns of DM and pyrethrin accumulation with FMI may contribute to differences in DM and pyrethrin yield responses to application of treatments such as water stress at different FMI stages.

The responses to water stress varied with flowering stage that treatments were applied. The FMS 4 stage treatment produced fewer numbers of flowers when compared to the control. This suggested the water stress applied at this stage reduced plant growth, presumably by reducing the rate of photosynthesis, leading to a reduction in the number of flowers and flowering stems. FMS 4 was found to be the stage of flower development where the effects of water stress could not be fully overcome by returning plants to field capacity before harvest time.

The DM yield of plants exposed to water stress at FMS 2 was similar to control, which was well watered. After the stress period the plants were put back on full water for about 20 days. This gave enough time to the plants to recover from the stress in terms of DM production. The low DM yield in the rest of the FMS stages suggested the plants had not recovered from stress in the period between the end of the treatment and harvest. The result was consistent to an earlier observation that crops may recover relatively quickly when re-hydrated following water stress early in flower development (Chung, 1987). A trend

for greater flower number in the well-watered control was compensated by smaller flower weight with same dry matter across all FMS treatments, again demonstrating the yield plasticity within pyrethrum.

The plasticity in yield formation responses noted in pyrethrum, combined with both dry matter accumulation and biosynthesis of secondary plant metabolites, the pyrethrins, contributing to crop yield, makes yield prediction difficult for this crop. It is perhaps not surprising that factors such as light interception, nitrogen and water stress that explain yield variability in many temperate crops (Hay and Walker 1989) only partially explain yield variability in pyrethrum. Separating the effects of treatments such as those examined in this study on the rates of pyrethrins synthesis and dry matter accumulation, and identification of relationships between production of these two yield components in pyrethrum, is needed to gain greater understanding of yield formation in the crop.

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